THE EFFECT OF DIFFERENT DIETS ON HAEMATOLOGY AND SERUM BIOCHEMISTRY IN DOGS

ELK Camilla Frisk

Licentiate thesis

Subject- Small Animal Medicine

Department of Equine and Small Animal Medicine

Faculty of Veterinary Medicine

University of Helsinki

2018

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Tiedekunta □- Fakultet – Faculty Faculty of Veterinary Medicine	Osasto □ - Avdelning – Department Department of Equine and Small Animal Medicine

Tekijä□□□ - Författare – Author
Camilla Frisk

Työn nimi□□ - Arbetets titel – Title
The Effects of Different Diets on Haematology and Serum Biochemistry in Dogs

Oppiaine □ - Läroämne – Subject
Small Animal Medicine

Työn laji□□ - Arbetets art – Level	Aika□□ - Datum – Month and year	Sivumäärä□□ - Sidoantal – Number of pages
Licentiate thesis	2018	44

Tiivistelmä□□ - Referat – Abstract

Feeding raw food has increased in popularity and many advocate the good effects of it. Only few studies on raw food has been done, mainly on negative effects such as the risk of infection when handling raw meat. Therefore, the purpose of this study was to investigate whether different diets, especially raw food, has an impact on blood parameters. The hypothesis was that raw food will have an impact on the blood parameters.

A total of 101 dogs were included in the analysis. Both hematologic and serum biochemical analyses were made. The owners were asked to fill in a questionnaire with information about their dog and the percent of each food they give to their dogs. Diets were defined as raw food, dry food, canned food and homemade food. Based on the questionnaire, the dogs were divided into different diet groups. Staffordshire bull terriers were also analysed individually since they consisted the majority of the population (n =80). The diet groups were as follows; 100 % raw food, 100% dry food and mixed diet. The population was also divided into 5 groups according to a set percent of either raw or dry food (1 = 0%, 2 = 1–30 %, 3 = 31–60 %, 4= 61–99 %, and 5 = 100 %). The mean values of the blood parameters in all groups were compared statistically (Kruskal-Wallis test).

Differences were found between raw and dry and raw and mixed diets. The blood values that most often differed were erythrocytes, haemoglobin, alkaline phosphatase (ALP), creatinine, cholesterol, sodium and protein. Erythrocytes, haemoglobin, protein and creatinine increased with increased amount of raw food. ALP and cholesterol showed the opposite. Sodium showed high values in groups with high amounts of raw food and low values in mixed diets.

This study gave evidence that diet is affecting blood parameters. In which extent it can affect remains unclear since no exact information about the diets were collected. Further studies need to be done to evaluate the real effect of a raw diet on blood parameters and whether it should be incorporated in clinical work.

Avainsanat – Nyckelord – Keywords
canine, haematology, biochemistry, blood, diet, raw food, dry food

Säilytyspaikka – Förvaringställe – Where deposited
HELDA- Helsinki University digital archive

Työn johtaja (tiedekunnan professori tai dosentti) ja ohjaaja(t) – Instruktör och ledare – Director and Supervisor(s)
Director: Docent Anna Hielm-Björkman, DVM, PhD
Supervisor: Johanna Anturaniemi, MSc (Animal Breeding), Academic agronomist

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Tiedekunta □- Fakultet – Faculty Veterinärmedicinska Fakulteten	Osasto □ - Avdelning – Department Avdelningen för klinisk häst- och smådjursmedicin	
Tekijä□□□ - Författare – Author Camilla Frisk		
Työn nimi□□ - Arbetets titel – Title Effekten av olika dieter i hematologiska och serum biokemiska analyser hos hund		
Oppiaine □ - Läroämne – Subject Smådjursmedicin		
Työn laji□□ - Arbetets art – Level Licentiatavhandling	Aika□□ - Datum – Month and year 2018	Sivumäärä□□ - Sidoantal – Number of pages 44

Tiivistelmä□□ - Referat – Abstract

Att ge råfoder till sina hundar har stigit i popularitet och flera förespråkar dess goda effekter. Negativa effekter som exempelvis infektionsrisken med rått kött har undersökts. Däremot finns det väldigt få vetenskapliga studier om råfodrets egentliga effekt på hundars hälsa. Därmed var syftet med denna licentiatavhandling att studera olika dieters inverkan på hundars hematologiska och serum biokemiska blodvärden. Av speciellt intresse var att studera effekten av råfoder på blodvärdena och om dessa värden avviker från utsatta referensvärden. Hypotesen för denna studie var att dieten har en effekt på hundars hematologiska och serum biokemiska blodvärden.

I analysen av diet och blodvärden deltog 101 hundar. Hematologiska och serum biokemiska parametrar mättes. Ägaren tillbads fylla i ett frågeformulär med information om hunden. Dieten var uppdelad i råfoder, torrfoder, konservfoder och hemlagad mat. Ägarna tillbads fylla i andelen procent de gav av ovannämnda foder. På basen av frågeformuläret grupperades de in i olika dietgrupper. Staffordshire bullterriers bildade även en egen grupp då de utgjorde majoriteten av populationen (n = 80). Grupperna var enligt följande; 100 % råfoder, 100 % torrfoder och de som åt en blandad diet. Populationen delades även in i 5 olika grupper enligt andelen % de åt av antingen råfoder eller torrfoder (1 = 0 %, 2 = 1–30 %, 3 = 31–60 %, 4 = 61–99 %, 5 = 100 %). Medeltalet av varje blodvärde i alla grupperna jämfördes statistiskt (Kruskal-Wallis test).

Statistiska skillnader i blodvärdena hittades mellan råfoder och blandfoder respektive råfoder och torrfoder. De parametrar som oftast skiljde sig var erytrocyter, hemoglobin, alkaliskt fosfatas (ALP), kreatinin, kolesterol, natrium och protein. Erytrocyter, hemoglobin, protein och kreatinin ökade då mängden råfoder som hundarna åt, steg. ALP och kolesterol visade omvänd trend. Natrium visade sig vara högt i grupper med mycket råfoder och lågt då man blandade foder.

Hypotesen för denna studie uppfylldes och vi kan konstatera att blodvärden skiljer sig statistiskt enlig förtärd diet. I hurdan utsträckning de skiljer sig och huruvida man borde ta i beaktande diet inom kliniskt arbete är ännu osäkert och kräver fortsatta studier.

Avainsanat – Nyckelord – Keywords
canine, haematology, biochemistry, blood, diet, raw food, dry food

Säilytyspaikka – Förvaringställe – Where deposited
HELDA- Helsingfors universitets digitala arkiv

Työn johtaja (tiedekunnan professori tai dosentti) ja ohjaaja(t) – Instruktör och ledare – Director and Supervisor(s)
Ledare: Anna Hielm-Björkman, DVM, PhD
Instruktör: Johanna Anturaniemi, MSc (Animal Breeding), Academic agronomist

CONTENTS

1 INTRODUCTION

The domestic dog is thought to have evolved from the wolf. Despite old beliefs of a direct ancestry to wolves, new studies show that the dog and wolf separated earlier than thought and from different wolf populations (Skoglund et al. 2015). While some digestive traits are still the same, e.g. the preference of lipid-rich diets, three specific genes have been found to have played an important role in the evolution and separation from wolves (Bosch et al. 2015). These three genes made starch digestion more efficient and is tought to have been a crucial step in the domestication process (Axelsson et al. 2013).

The thought of feeding pets a diet more in resemblance to the diet of wolves has risen in popularity (Beloshapka et al. 2012). The concept of raw food has emerged and with it questions about its adequacy and safety. A few studies have been evaluating the risks and benefits of a raw food diet. Weese et al. (2005) presents potential bacteriological risks such as salmonella, whereas Beloshapka et al. (2012) concludes that raw meat-based diets are very digestible and results in low faecal volume. In Finland the risk of salmonella is low because of a national salmonella control programme, comprising control over cattle, poultry, swine, eggs, and meat. The goal is to keep the incidence below 1 %, which has been achieved so far (Evira). Therefore, the real health risks of raw diets remains to be determined.

Furthermore, the potential effects of different diets, especially raw food diets, on blood parameters has been poorly investigated. Most studies investigate the effects of age, breed and gender on blood parameters (Lawrence et al 2013, Uhrikova et al. 2013, Chang et al. 2016). Since blood parameters are an important tool in disease diagnostics (Chang et al. 2016), it is important to know what might influence the values.

In Finland, we define raw food as an unprocessed, low-carb, medium or high protein, and medium or high fat diet. It can be composed from foodstuffs such as red or white meat, egg, bone, cartilage, vegetables, fruits, and berries. Dry food we define as at least heat processed, sometimes even extruded food that are usually high in carbohydrates, medium or low in protein, and low to medium in fat. Highly processed ingredients, such as bone meal, starch, and meat meal, are most often used in dry foods. Mixed food is defined as all food that cannot be put into the two categories mentioned above, or mixing those two with each other or with foods mentioned below. Ingredients often

used in mixed foods are canned dog food, minced meat (either raw or cooked), rice, porridge, and processed human diets (e.g. liver casserole, food leftovers, cooked vegetables, and dairy products). Based on data from the 12.000 dog DogRisk questionnaire (personal communication, Anna Hielm-Björkman) mixed food is suspected to be high in carbohydrates.

Based on this background, the objective of this study was to determine the effects of diet on haematology and serum biochemistry in client-owned dogs. Of special interest was the effect of feeding raw food as a sole diet and as a part of a mixed diet. Regarding the small amount of research done on this topic, we also wanted to contribute with more information to those few studies already done.

The hypothesis of this study was that feeding dogs a diet based on raw food will affect at least some haematological and biochemical values.

2 LITERATURE REVIEW

2.1 Evolution of *canis familiaris*

The exact time when dogs diverged from wolves is thought to have been about 11 000–16 000 years ago (Freedman et al. 2014). Though, a more recent study has recalibrated the estimated divergence to be a lot earlier, about 33 000 years ago (Wang et al. 2013). This is in line with another study that estimated the time of divergence to be about 27 000–40 000 years ago (Skoglund et al. 2015). Whichever the case, all three studies indicate an earlier divergence of dogs from wolf populations than thought. Furthermore, all three studies agree on the domestication process consisting of more than just one event and that there has been gene flow between wolves and dogs during this long process (Freedman et al 2014, Skoglund et al. 2015, Wang et al. 2013). Evidence from studies by both Freedman et al. (2014) and Wang et al. (2013) supports the thought of a single domestication location. This is in contrast with the study by Skoglund et al. (2015), who suggests that domestication happened from many wolf populations from different regions (Skoglund et al. 2015). However, Wang et al. (2013) found high genetic diversity among dogs from southern East Asia, suggesting that the initial divergence from wolves happened there. The same study also suggests the Chinese indigenous dog to be an intermediate form between today's gray wolves and modern breed dogs, and that the divergence between Chinese indigenous dogs and modern breed dogs happened about 15 000 years ago, when a group of dogs migrated to other parts of the world (Wang et al. 2013).

Irrespective of the background, the course of domestication can be divided into three periods; pre-domestic ancestry, early domestication, and modern artificial selection (Hewson-Hughes et al. 2013). A similar study also concludes the same three steps (Wang et al. 2013). Pre-domestication took place during the Paleolithic era (until about 12 000 years ago), when wolves started taking advantage of humans in forms of stealing hunts or taking advantage from an unsuccessful hunt leaving a wounded animal an easy prey (Bosch et al. 2015). This coevolution between wolves and nomads was probably due to spontaneous events but would later lead to conscious selection by humans (Hewson-Hughes et al. 2013).

When humans started their transition from a hunter-gatherer style to agriculture, wolves followed and became a target for natural selection (Hewson-Hughes et al. 2013). Agriculture made way for a new food niche; food waste products (Bosch et al. 2015). Early domestication, with a new food source and close coexistence with humans, favoured specific traits in wolves (Axelsson et al. 2013). Besides dietary genetic changes other traits like less aggressiveness, smaller body size, and better social cognition were enhanced (Axelsson et al. 2013). Wolves slowly became adapted to humans and during the Neolithic period (about 10 000-4000 years ago) humans started to domesticate wolves intentionally. This was done through selective breeding and interbreeding with wild wolves (Bosch et al. 2015). Further genetic analysis has shown that similar genes (genes related to metabolism, neurological processes and cancer) in both dogs and humans have been under positive selection during this time, strengthening the thought of a parallel evolution between humans and dogs (Wang et al. 2013).

The third step, i.e. selective breeding, has continued up to this day. During the last 200 years dogs have been a target for very specific selection, giving way for over 400 phenotypically diverse breeds (Hewson-Hughes et al. 2013). Today dogs differ significantly from wolves in many ways. The genetic changes are not yet thoroughly investigated but 36 genomic regions are said to have been a target for selection, most of them potentially affecting behavioural traits. Morphological changes can also be seen, such as smaller teeth, brain, and skull size (Axelsson et al. 2013). However, dogs and wolves still share about 40 % of single-nucleotide polymorphism found in the genome of both dogs and wolves (Wang et al. 2013).

2.1.1 The dietary evolution of dogs

Actual domestication has taken place during the last 10 000 years (from the beginning of the Neolithic period until today), which some believe is a short time for the evolution to make major changes in metabolism and physiology (Frassetto et al. 2009). There are, however, studies that show differences in macronutrient selection between wolves and dogs (Hewson-Hughes et al. 2013) and gene-differences coupled to metabolism (Axelsson et al. 2013).

Wolves are regarded as true carnivores who choose a macronutrient profile of 54 % protein, 45 % fat, and 1 % carbohydrate (Bosch et al. 2015). Dogs, however, prefer a

protein: fat: carbohydrate ratio of 30: 63: 7 % (Hewson-Hughes et al. 2013). This might be partly reflected in the differences between food items consumed. Modern wild wolves prefer consuming ungulates like moose and deer whereas dogs, as stated earlier, evolved from wolves with traits specifically adapted to eating more vegetal matter besides animal items (Bosch et al. 2015). Regarding the preference for lipids, there are different opinions whether it has developed during early domestication or already from wolves (Hewson-Hughes et al. 2013, Bosch et al. 2015).

Wolves have evolved to survive on a so-called feast and famine lifestyle and can, when big prey is available, consume up to 22% of their body-weight during one meal (Bosch et al. 2015). Their stomach is also capable of extension, which is necessary when eating large amounts of food. Other traits necessary for wolves are the ability to recover from weight loss and cope with longer periods of low food intake. Synthesis of nutrients and downregulation of protein catabolism minimizes metabolic losses, which is essential during periods of famine (Bosch et al. 2015). Dogs have been shown to have the same traits, further evidencing that domesticated dogs still resemble wolves in many ways. For example, dogs are shown to efficiently use ketone bodies as an energy source when energy intake is low (Bosch et al. 2015). They also seem to eat more than their daily energy need, if offered food *ad libitum* (Hewson-Hughes et al. 2013), indicating that though their lifestyle has changed they still reflect their ancestors in some aspects.

The domestic dog is nowadays in many contexts classified as omnivorous because of distinct traits that can't be found in obligate carnivores. These traits are characterized by a slow protein catabolism, increased glucose use, endogenous synthesis of niacin, increased glucokinase activity, and increased capacity for starch digestion and absorption (Bosch et al. 2015). However, some similar traits that reflects the carnivorous lifestyle are also still seen in dogs. These are for example a lack of amylase in salivary glands, a short digestive tract, and the capability to conjugate taurine with bile acids (Bosch et al. 2015). The next section will discuss the genetic background to one of these traits, namely the increased utilisation of glucose.

2.1.2 Genes connected to starch-adaptation

The new food niche during the Neolithic era favoured wolves with three mutational genes related to metabolism. These genes gave wolves more efficient starch digestion and an

opportunity to better utilize energy from carbohydrates (Bosch et al. 2015). In a large study of gene differences between dogs and wolves, ten genes whose functions were coupled to starch and fat metabolism where found. Of those ten genes, alpha-amylase 2B (*AMY2B*), maltase-glucoamylase (*MGAM*), and sodium/glucose cotransporter-1 (*SGLT1*) represents the three stages of starch digestion, and were found to differ between dogs and wolves (Axelsson et al. 2013). The *AMY2B* gene, responsible for producing alpha-2B-amylase, has undergone copy number change which means that dogs have more copies (4–30) of the gene than wolves, which only have two (Axelsson et al. 2013). Amylase is the main enzyme in the first step of starch digestion (Figure 1), cleaving starch into maltose and oligosaccharides (Axelsson et al. 2013).

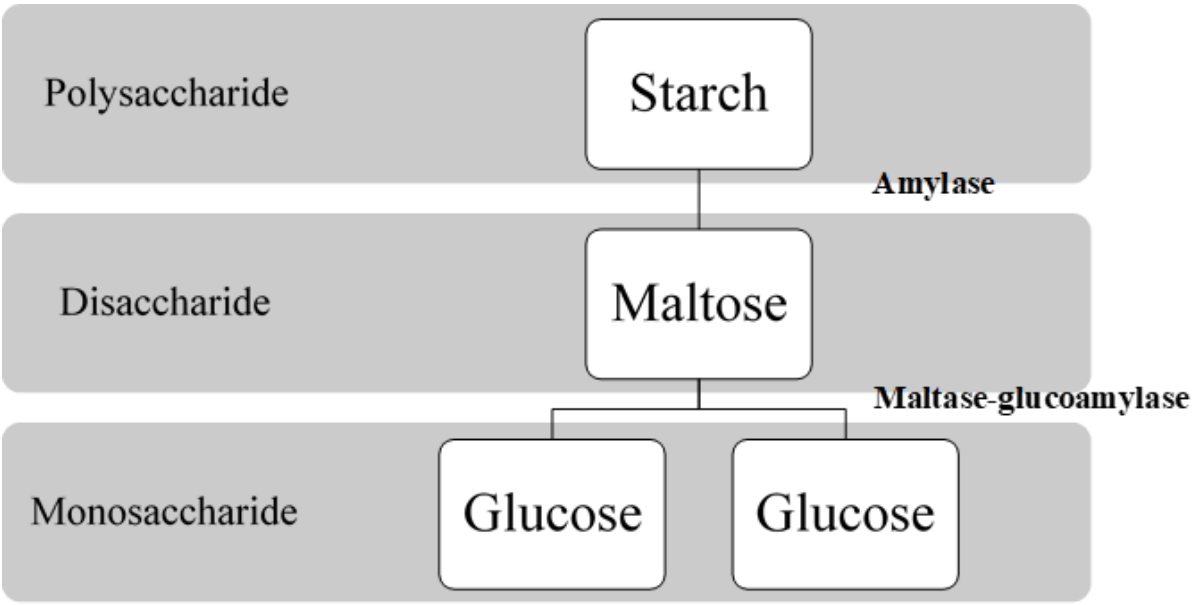

Fig.1 A simplified figure on starch digestion. In bold are the necessary enzymes for each step.

The number of copy number variation (CNV) affects amylase activity with more copy number variation leading to an increase in activity and thus a more efficient starch digestion (Arendt et al. 2014). The gene expression was shown to be higher in dogs compared to wolves, further increasing amylase activity (Axelsson et al. 2013). The importance of this gene has been evaluated further concluding that CNV also differs widely between breeds (Arendt et al. 2014) and especially between breeds with different starch intake (Reiter et al. 2016). Those who consumed a diet rich in starch carried more CNV than breeds consuming a low-starch diet (Reiter et al. 2016). Specific breeds like Samoyeds and Greenland Sledge dogs usually have only two copies of the gene whereas German Shepherds and Beagles show more variation in the gene copy number (Arendt et

al. 2014). Also Siberian Huskies have shown to have less CNV than other breeds (Freedman et al. 2014). Common to these breeds is that they come from the very north hemisphere, where the people mainly lived as hunter-gatherers instead of agriculturalists. In other words, their diet has consisted of more protein than carbohydrates, further strengthening the results by Reiter et al. (2016).

Another difference was seen when studying the *MGAM* gene's haplotypes. The *MGAM* codes for the enzyme maltase-glucoamylase which is important in the second step of starch digestion (Axelsson et al. 2013). The enzyme catalyses the hydrolyse reaction of maltose, converting it into glucose. In the study by Axelsson et al. (2013) instead of finding CNV, they found at least one haplotype in most of the dogs, most of them being homozygous for it (55 dogs out of 68). The same haplotype was absent in wolves (Axelsson et al. 2013). The third gene, *SGTL1*, codes for the sodium/glucose cotransporter-1 in the small intestine and is necessary for the absorption of glucose through the luminal wall. It represents the last stage of starch digestion (Axelsson et al. 2013). The same study by Axelsson et al. (2013) found one common haplotype for *SGTL1* in all 71 dogs but only in one wolf out of 19 tested. Of the dogs, 63 were also homozygous for the haplotype (Axelsson et al. 2013).

Even though wolves and dogs are closely related, these genes indicate that dogs have adapted to a more omnivorous lifestyle with a diet different from wolves (Freeman et al. 2013, review). This has probably been the case because of a parallel evolution between dogs and humans and the increased need of efficient starch digestion due to the new diet composition (Axelsson et al. 2013).

2.2 Haematology and serum biochemistry of dogs

Haematology is the examination of blood cells whereas blood biochemistry examines the components in blood plasma or serum. Blood cells include erythrocytes, leukocytes, and thrombocytes, and they are usually measured in cell counts or volume. The molecule haemoglobin is also included in hematologic measurements (In the book by Harvey 2012). Blood biochemistry is done on either serum or plasma. Plasma is the fluid component of blood and consists of water, lipids, proteins, carbohydrates, vitamins, minerals, and inorganic salts. Plasma samples still contain the coagulating component fibrinogen because it is prevented from coagulating. Serum is obtained from the fluid that

remains after the blood has coagulated and been centrifuged. Therefore, serum samples often contain lower amounts of protein than plasma samples (In the book by Harvey 2012).

Hematologic and biochemical values are important measurements when evaluating the health of the patient. When assessing blood components both physiological and pathological changes can be seen indicating disease or other abnormalities (Khan et al. 2011). Tests may therefore help noticing subclinical disease or help diagnose diseases with non-specific symptoms. Evaluation of the effect of medication can also be done with hematologic tests (In the book by Harvey 2012). It is important to have general information about the patient under testing because individual variations may lead to wrong assumptions (Choi et al. 2011). A value can be read as a pathologic finding though it is not, or a pathologic state might go unrecognized because of specific breed differences (Nielsen et al. 2010). Therefore, it is necessary to accurately evaluate the results and have reliable reference intervals (Choi et al. 2011). Reference intervals are best done on a big heterozygous group of healthy individuals. The more heterozygous the population the more variation you get in the reference intervals and the value become more adaptable to different patients. It is also important to remember that, normally, 95% of the population will be within reference intervals whereas 5% will be either above or under. Therefore, small deviations from the reference interval doesn't necessarily mean that the individual is unhealthy (In the book by Harvey 2012).

Even though reference intervals are done on a big population of dogs, variations are still evident (Uhrikova et al. 2013). These variations may be due to age, breed, sex, genes (Lawrence et al. 2013), or diet (Swanson et al. 2004). In order to establish more specific reference values, many studies have been done researching different factors affecting these values (Swanson et al. 2004, Pasquini et al. 2008, Lawrence et al. 2013). The most common factors studied are breed, sex, age, and diet.

2.2.1 Breed

There have been a few studies that shows a need for breed-specific reference intervals (Nielsen et al. 2010, Uhrikova et al. 2013). A study of different sighthound breeds discovered that some of the haematological and biochemical values differed from general reference values (Uhrikova et al. 2013). The study noticed higher haemoglobin (HGB),

red blood cell count (RBC), mean cell haemoglobin concentration (MCHC), and haematocrit (HCT) in all sighthound breeds (Whippet, Greyhound, Italian Greyhound, Sloughi, Saluki, Borzoi, Pharaoh Hound, and Azawakh). Mean cell volume (MCV) values were above reference intervals in Whippets and Greyhounds. Higher alanine aminotransferase (ALT) values were noticed in Italian Greyhounds whereas calcium levels were below reference values in all breeds but Pharaoh Hounds (Uhrikova et al. 2013). Torres et al. (2014) recognized abnormal hematologic values in Dachshunds compared to dogs of mixed breed. They found that Dachshunds had higher HCT and HGB values than mixed breed dogs. These values were higher because of the high RBC counts that also were found in Dachshunds (Torres et al. 2014). In Bernese Mountain dogs, researchers found differences in alkaline phosphatase (ALP), ɣ-glutamyltransferase (GGT), MCHC, cholesterol, and total bilirubin values compared to the laboratory's standard reference intervals. GGT, MCHC, and cholesterol had reference intervals shifted upwards whereas total bilirubin had lower values. ALP showed a wider reference range than the standard reference ranges used in this study (Nielsen et al. 2010). Chang et al. (2016) noticed significant differences in total protein, albumin, sodium, potassium, calcium, phosphate, cholesterol, bilirubin, ALP, ALT, and creatinine between individual breeds and mixed breed. He proposes that, especially for creatinine, there should be breed specific reference intervals (Chang et al. 2016).

Contrary to the study results above, Lavoué et al. (2014) didn't find any breed-specific values in Dogues de Bordeaux that differed significantly from standardized reference intervals. Some values such as HGB, HCT, MCV, and MCHC were higher than reference intervals but not enough to be clinically relevant (Lavoué et al. 2014). Despite the results, Lavoué et al. (2014) did conclude that some breed-specific reference intervals may be of importance in breeds with specific diseases that can be detected through hematologic tests, such as polycythemia in Dogues de Bordeaux.

Although studies show that differences between breeds are relevant, the study population is often too small to make reliable conclusions (Uhrikova et al. 2013, Torres et al. 2014). Therefore, new breed-specific reference intervals cannot be reliably determined and further studies should be done with a wider study population (Uhrikova et al. 2013).

2.2.2 Sex

The impact on sex and neutering status have also been evaluated to some extent. Pasquini et al. (2008) found that gender does affect some metabolites such as total cholesterol and high-density lipoprotein cholesterol, both values being higher in intact females than in intact males (Pasquini et al. 2008). According to a study of both neutered and intact dogs, serum level of vitamin D was shown to be higher in intact male dogs compared to intact female dogs. Neutering status decreased the serum concentration of vitamin D significantly in males but not in females (Sharp et al. 2015). Another large study of 6046 dogs showed that sex did affect, among other things, HGB, mean cell haemoglobin (MCH), MCHC, and white blood cell count (WBC) (Lawrence et al. 2013). WBC concentrations were lower in females than in males, whereas neutering status further affected HGB, MCH, MCHC, and WBC. Neutered dogs had increased HGB, MCH, and MCHC values whereas WBC and thrombocyte counts were lower compared to intact dogs (Lawrence et al. 2013). In contrast, a study of 14 German Shepherds didn't show any differences in haematological values between sexes (Olayemi and Ighagbon 2011). Effects of gender on serum biochemistry parameters has been poorly investigated. One large study, with over 3000 dogs, found no differences between sexes in albumin, calcium, sodium, glucose, urea and phosphate concentrations. Total protein and creatinine differed between female and male dogs (lower in female than male dogs) as well as between neutered and intact dogs. Total protein showed lower values in neutered dogs whereas creatinine was higher in neutered dogs, compared to intact dogs. Also, ALP differed between neutered and intact dogs, being higher in neutered than in intact dogs (Chang et al. 2016).

2.2.3 Age

Differences in reference values between dogs of different age is something many researchers have considered. In one study all haematological values but MCHC were found to vary with age. For example, thrombocyte count were shown to increase with age whereas erythrocyte count increased up to about 3 years of age after which it started to decrease with age (Lawrence et al. 2013). Harper et al. (2003) studied the effects of breed and age on Beagles and Labrador retrievers with the result that young dogs (under 1 year of age) had the most noticeable differences. WBC and MCV values decreased with age whereas RBC, HGB, and HCT values increased during the first year of life after which it

reached a steady level of concentration. Plasma calcium concentrations varied with age, being the highest between the ages of 3–8 weeks. ALP increased during the first 16 weeks of life after which it started to decrease and stabilize. Total protein concentrations increased up to one year of age, after which it became stable. As a conclusion, young dogs differed in some haematological and biochemical values but matured between 6–12 months of age when reaching adult values (Harper et al. 2003).

A more recent study of young dogs (of age 16–60 days) found lower values for RBC, HGB, HCT, albumin, total protein, and creatinine compared to adult dogs (Rørtveit et al. 2015). They also found that ALP, phosphate, calcium, and potassium levels were higher in puppies than in adults (Rørtveit et al. 2015). Another study evaluating the differences between young and geriatric dogs also found that values differed more from the average reference values in young dogs than in the old dogs. The young dogs had higher levels of glucose, calcium, phosphorus, potassium, and ALP (Swanson et al. 2004). On the contrary, Chang et al. (2016) found that potassium increased with age whereas glucose did not significantly differ with age. Other parameters such as total protein, albumin, calcium, and phosphate seemed to follow the same curve as previous studies show (Chang et al. 2016). Whatever the results, all studies indicate a possible need for age-specific reference intervals, especially when assessing young dogs under one year of age.

2.2.4 Diet

Hematologic and biochemical values are known to be affected by diet (Harper et al. 2003), something which is of great interest to many researchers. Many studies on the topic has been done, varying from evaluating diets with different composition to exclusion of necessary nutrients (Davenport et al. 1994, Swanson et al. 2004).

A diet with low amounts of protein can be detected using blood analysis. A study on the effects of low-protein diets on dogs' blood metabolites showed that biochemical values coupled to protein metabolism either increased or decreased due to protein malnutrition (Davenport et al. 1994). For example, the albumin and total protein concentrations decreased below normal ranges whereas ALP concentrations increased over the upper reference range when feeding a protein deficient diet (Davenport et al. 1994). A study on stray dogs showed that they had lower mean glucose and cholesterol concentrations compared to normal reference ranges. This could indicate a possible effect of an

inadequate diet intake on blood measurements (Khan et al. 2011). These findings suggest an importance of considering diet in a clinical assessment (Davenport et al. 1994). Kronfeld et al. (1977) studied the effects of carbohydrate on sled dogs and found that dietary carbohydrate is not necessary for dogs to consume. Three diets were evaluated; one with zero carbohydrate, one with medium, and one with high concentrations of carbohydrates. The glucose levels remained stable regardless of diet. Dogs consuming the zero carbohydrate diet did not show any adverse effects such as ketosis or hypoglycaemia (Kronfeld et al. 1977). The zero-carbohydrate group were also able to maintain some important serum metabolite concentrations during training, making them less susceptible to for example hypocalcemia or hypovolemia during training (Kronfeld et al. 1977).

Even though diet does have an impact on blood metabolites, the values usually stays within reference ranges. A study comparing a normal (control) diet with a high endurance diet (more protein and fat) on dogs undergoing hard training, didn't find any abnormal haematological values (de Godoy et al. 2014). However, they did find differences in serum biochemistry concentrations between diets. The differences were found between phosphate, blood urea nitrogen, chloride, cholesterol, and carbon dioxide concentrations. The parameters where higher when feeding the high endurance diet, except for cholesterol which was lower compared to the control diet (de Godoy et al. 2014). Cholesterol is well known to be affected by diet. A diet rich in fish and fish by-products is shown to decrease cholesterol and high-density lipoprotein concentrations (Pasquini et al. 2008). Another study also found differences in cholesterol concentrations when comparing two different diets. Cholesterol concentrations were higher in dogs fed an animal product-based diet than in dogs fed a plant-based diet (Swanson et al. 2004). However, other blood parameters did not differ significantly, proposing that if the diets' nutritional requirements are met, blood metabolites will not be affected (Swanson et al. 2004). This is in correspondence with a study comparing raw-meat based diets with slightly different content (Beloshapka et al. 2012). The diets contained either beef or chicken and where supplemented with either inulin or yeast cell-wall extract. Regardless of composition, they showed no variations in blood metabolites (Beloshapka et al. 2012).

2.3 The impact of diet on health

Nutrition and diet can have an influence on many diseases. The World Small Animal Veterinary Association (WSAWA) has therefore taken note of this and proposes that veterinary practitioners should evaluate their patients' diet as a part of their standard physical examination (Chandler and Takashima 2014, review). Inadequate nutrition, like nutrient deficiencies, may develop disease whereas some diseases (i.e. kidney disease and osteoarthritis) can be partly treated with a specific diet (Chandler and Takashima 2014, review). The effects of diets on diseases has been variably studied and here follows a few examples.

Two different studies investigating copper-associated hepatitis in Labrador retrievers showed that copper accumulation in the liver can be minimized using diets with low copper- and high zinc content. In this case, diet could work as a sole treatment to the disease (Hoffman et al. 2009, Fieten et al. 2014). Diarrhoea is usually treated with a specific diet, either alone or with accompanying treatments (Wennogle et al. 2016). A study comparing two commercial diets specifically developed for dogs with gastrointestinal disorders, found that both diets decreased the occurrence of acute diarrhoea in dogs. The study concludes that dietary treatment can be the sole therapy, however, depending on the underlying cause of the diarrhoea (Wennogle et al. 2016).

Obesity has increased in pet dogs following an increase in other disorders coupled to obesity, such as diabetes mellitus (Peña et al. 2014). Obesity is often treated with dietary restriction, exercise and sometimes medical treatment. However, a study found that medical treatment would be unnecessary in a weight loss programme if the dietary restriction is well executed (Peña et al. 2014). A carefully made weight loss programme is very important because calorie restriction, as in reducing the amount of food, easily leads to nutrient restriction and nutritional deficiencies (Linder et al. 2013). It is also important to lose weight safely without losing too much fat-free mass. This is ensured e.g. with a high protein-content (Diez et al. 2004). Therefore, pet food companies have developed specific diets high in nutrients and low in energy to ensure a safe weight loss (Linder et al. 2013).

More specific disorders, such as Canine Epitelioid Cramping Syndrome, may also be treated with a specific diet. Researchers has found that gluten is linked to the disease and that a gluten-free diet could reduce the symptoms in some dogs (Lowrie et al. 2015). Also

for dogs with diabetes, diet can be a useful treatment tool. A study of healthy dogs and the effect of diet on postprandial glucose concentrations showed that a diet low on carbohydrate led to lower postprandial glucose levels compared to diets with higher carbohydrate content (Elliott et al. 2012). Although the study was made on healthy dogs, the results suggest a positive effect of a low-carbohydrate diet in diabetic dogs' postprandial glucose levels. This could possibly mean that less additional insulin doses would be needed (Elliott et al. 2012).

2.4 Commercial pet food

The commercial pet food industry provides many different pet foods to meet every pets' nutritional need, every pet owner's idea of a perfect dog diet, and every owner's will to spend. Traditionally, pet food has been produced to meet specific nutrient requirements without thinking of ingredients (Buff et al. 2014, review). Although with humans preferring foods that mirror their own diet, pet food industries have started advertising "natural" pet foods. The term "natural" is defined by the European Pet Food Industry Federation (FEDIAF) as pet food components with no added items and which, though processed, maintain their natural composition. However, proponents of natural pet food also want the ingredients to be "whole", meaning that instead of natural ingredients grounded into meal it has to be whole vegetables and pieces of meat (Buff et al. 2014, review). Therefore, the composition and processing of conventional pet foods and natural whole foods will differ from each other.

Processing of foodstuff leads to physical changes in for example protein and can therefore affect digestibility and absorption (Freeman et al. 2013, review). Both negative and positive changes can be seen. Heating of animal protein can lead to amino acid loss whereas digestibility of plant protein usually benefits from heating (Freeman et al. 2013, review). A study comparing the creatine (a component of skeletal muscle) and creatinine concentrations in processed and unprocessed foods found that unprocessed diets (e.g. raw meat diets) had higher creatine and lower creatinine levels compared to processed foods (e.g. dry food diets) (Dobenecker and Braun 2015). These results further support the thought that processing affects the foodstuff's nutritional content (Dobenecker and Braun 2015). Furthermore, processing of food is regarded a safety measure, decreasing the risk of contamination and possible infection (Buff et al. 2014, review). Yet, the thought of

natural pet food is dominating and has led to the increasing popularity of feeding so called raw food (Beloshapka et al. 2012). The concept of raw food and why it has been coined will be discussed in the following chapter.

2.5 Raw food

The popularity of feeding raw food to dogs has increased during the last years (Freeman et al. 2013, review). This is partly because of humans' approach to food and feeding time. For humans, food is not only seen as a necessity, it is a social event in which the pet is nowadays included (Schlesinger and Joffe 2011, review). Feeding animal by-products to a family member does not sound palatable, nor eating the same food every day, which has led to owners favouring homemade diets that usually are more variable (Schlesinger and Joffe 2011, review). Other reasons are because of distrust to pet food companies (Stockman et al. 2013), possible health benefits, and the desire to feed dogs a more natural diet (Weese et al. 2005).

Raw meat-based diets consist of uncooked ingredients derived from animals such as bones, organs, skeletal muscles, eggs, and milk (Freeman et al. 2013, review). These foods usually contain a high protein concentration and a low carbohydrate concentration (Buff et al. 2014, review). Raw food diets can be home-made or produced by a pet food company (Freeman et al. 2013, review). Commercial raw food diets are often frozen or fresh mixtures of different components, to ensure a complete and balanced diet. There are also commercial products meant to be supplements to the pets' diet (Freeman et al. 2013, review). Recipes for home-made diets can be written by veterinarians, owners, or other non-veterinarians (Stockman et al. 2013). In a study comparing recipes found from books or internet, they found that very few of the recipes made for a complete and balanced diet (Stockman et al. 2013). As earlier stated, an unbalanced diet can cause disease in animals, leading to concerns about the adequacy of home-made diets. Home-made diets are usually executed through a rotation of different ingredients to meet a balanced diet over a longer period (Freeman et al. 2013, review). However, when analysing seven home-made recipes meant for rotation researchers found that the deficiencies in the recipes did not compensate each other in order to reduce the deficiencies (Stockman et al. 2013).

There are only few actual studies evaluating the risks or benefits of feeding raw diets (Freeman et al. 2013, review). The following sections will discuss the risks and benefits that we know of today, both subjective opinions and study results.

2.5.1 Benefits

Most of the benefits known are owners' experiences of feeding their own pets with some kind of homemade or commercial raw food diet. These benefits include better coat health, better palatability, better breath and faecal odour (Freeman et al. 2013, review). Other dubious benefits recorded are better immune function, control of allergies, and better overall health (Weese et al. 2005). Cats fed raw food were shown to have increased lymphocyte and immunoglobulin production. However, these cats also shed salmonella in their faeces, making it questionable whether the increase is due to infection or a direct health benefit from the diet (Freeman et al. 2013, review). In cats, feeding a raw meat-based diet has shown that faecal output decreases and that digestibility is better compared to feeding a dry food diet (Kerr et al. 2012). A similar study has been done on dogs showing that raw meat-based diets gave high nutrient digestibility, good faecal consistency, and normal blood metabolite values (Beloshapka et al. 2012). Heterocyclic amines have been associated with cancer when consumed in excessive concentrations. They are formed when muscle meat is heated and even though pet foods usually have low concentrations, mutagenic activity may still be evident (Freeman et al. 2013, review). Raw food is not heated and would therefore be safer considering this aspect.

2.5.2 Risks

The risks of feeding raw food has been evaluated in many studies. The most common concern is the contamination of pathogens causing a potential health risk for both owner and pet (Weese et al. 2005). A study analysing twenty-five commercial raw diets found coliform-bacteria in all diets, *Escherichia coli* in 64% of the diets and Salmonella in 20% of the diets (Weese et al. 2005). Another study found salmonella (*S.enterica*) when analysing 17 samples derived from raw meat products (Strohmeyer et al. 2006). The study also compared the level of contamination in commercial canned food versus raw food, confirming that canned food is less contaminated than raw food products (no statistical analyses were performed) (Strohmeyer et al. 2006). Some dogs, feeding on a raw food

diet, proved to shed salmonella in their stools. This further highlights the risk of contamination in raw feedstuff (Joffe and Schlesinger 2002). Salmonella infections in dogs tend to not develop clinical salmonellosis, but because it is usually easily colonized, the risk of salmonellosis is still high (Finley et al. 2007).

Even though no findings of clinical salmonellosis have been found in dogs, cats have been diagnosed with salmonellosis (Stiver et al. 2003). In a case report of two cats, necropsy and diagnostic testing showed that they both were infected with salmonella. The cats were from the same household and were fed a raw meat-based diet. The diet from case no. 2 underwent bacteriological testing and showed to be positive for the same salmonella strain (*S.newport*) that were found in the cats internal organs. However, diet samples from the first case were not available (Stiver et al. 2003).

Other bacteria than Salmonella has also been found in dog stools. A study investigating the prevalence of Campylobacter spp. in dog faeces, found that *Campylobacter upsaliensis* was the most common species found in the stools of the study population (Olkkola et al. 2015). *Campylobacter upsaliensis*, as well as salmonella, is a zoonosis, which causes gastroenteritis in humans. Because of the prevalence of the bacteria in dog stools, dog owners are considered to be at risk for infection. However, when the study investigated whether feeding raw food or not had an impact on bacterial findings, they found no correlation (Olkkola et al. 2015). A more recent study did find a correlation between campylobacter findings in raw feedstuff and fecal samples from dogs fed raw food. They also found campylobacter more frequently from dogs feeding on raw food than in dogs feeding on dry food. Although campylobacter is a common finding in dogs overall and not all campylobacter strains cause disease in humans, the real risk still remains uncertain and people at high risk for infection are recommended to be careful when handling raw food (Fredriksson-Ahomaa et al. 2017).

Other risks constitute nutritional deficiencies in homemade diets. Animal-based ingredients may vary significantly in protein and fat content, indicating the importance of thoroughly formulating the diet so that nutrient requirements are met (Beloshapka et al. 2012). A study comparing the nutritional content of 129 veterinarian recipes and 71 non-veterinarian recipes found that all but five recipes did not provide all the essential nutritional concentrations recommended by the National Research Council (NRC) or the Association of American Feed Control (AAFCO). The recipes were either deficient of some nutrients (e.g. vitamin D and zinc) or exceeded recommended concentrations.

Thirteen recipes even had ingredients considered toxic such as garlic and onion in them (Stockman et al. 2013). A lack of or an excess of nutrients may lead to so called diet-induced diseases. Diet-induced diseases are diseases that develop due to either deficient levels or exceeding levels of one or more nutrients. They are often caused by unbalanced homemade diets (Chandler and Takashima 2014, review). For example, vitamin D deficiency can lead to skeletal problems in puppies (Stockman et al. 2013).

Lastly, raw food diets containing bones can also pose a risk for fractured teeth and perforation of the gastrointestinal tract (Freeman et al. 2013, review).

3 AIMS OF THE STUDY

The aim of the study was to evaluate whether different types of diets would have an impact on hematologic and biochemical values and whether these possible differences would affect clinical interpretation. Of special interest was the effect of feeding different amounts of raw food with a mixed diet.

We also wanted to contribute to the research made on different diets and their effect on haematology and biochemistry.

4 MATERIALS AND METHODS

4.1 Study design

This study was composed of two different studies, but the execution of the study methods was the same. It was done by collection of blood samples for analysis and collection of information about the dogs through a questionnaire. The questionnaire (Appendix I) was given to the dog owners either in paper form or via internet. The owners were asked to fill in the following information about their dog or dogs: general signalment, living habitat, health status, medication, diet (percentages of dry, raw, homemade, and canned foods were given), dietary supplements, exercise habits and information about the dog's relatives' health status. The questions were in Finnish.

Inclusion criteria for participating in the study were owners filling in the questionnaire adequately and enough blood being drawn from the dog to complete analysis. Exclusion criteria were based on diseases affecting blood parameters; in this study, being cancer and hypothyroidism. There were no limitations regarding age, gender or breed.

The majority of the study population was collected during dog shows. Information about the study was sent out beforehand and the questionnaire was given to the owners after blood sampling. The other part of the study population was taken from a previous study consisting of 41 dogs of the same breed (Staffordshire bull terriers). These dogs had their blood collected twice but only the results from the latter collection was taken into this

study. Owners brought their dogs to the Veterinary Teaching Hospital of the University of Helsinki for blood sampling and they were given the questionnaire beforehand. This study was approved by the Animal Expert Board in Finland (ELLA, permit number: ESAVI/3244/04.10.07/2013).

Data from the questionnaire and the blood analyses were transformed into an Excel sheet for each dog (data not shown).

The following dietary supplements were included; animal fat, vegetable fat, vitamin C, vitamin B, vitamin D, vitamin E, calcium, zinc, magnesium, joint supplements, *lactobacillus* bacteria and seaweed. Habitat was defined as city, urban area or countryside. The dogs' health status was divided into four groups; healthy, diseased (=other than atopic), atopic and both diseased and atopic. The information about medication were divided into regular medications and medications given within the last three months. The different medications were also divided into groups according to their function (NSAID, antiparasitics, antibiotics, corticosteroids and gastroprotective drugs). Other parameters included were gender (both neutered and intact), age, breed and duration of fasting before blood sampling.

For statistical analysis, the study population was divided into three different groups depending on their diet composition. Dogs feeding on 100 % raw food formed one group, dogs feeding on 100% dry food formed the second group and the rest of the dogs, eating a mixed diet, formed the third group. The population was also divided into groups of how long they have been eating their current diet. Dogs who had been eating their current diet for longer than 3 months constituted a group whereas those who had changed their diet in the last 3 months formed the other group. For the statistical analyses we looked at the diet being fed at the time of this study.

Further statistical analyses required redistribution of the study population. Except for the primary 100 % diet groups including the whole study population, the same division were made for only Staffordshire bull terriers and for all dogs but Staffordshire bull terriers. The whole study population was also divided into 5 somewhat even groups according to the percentage of raw food being fed. The group distribution was as follows; group 1 feeding on 0 % raw food, group 2 feeding on 1–30 % raw food, group 3 feeding on 31–60 % raw food, group 4 feeding on 61–99 % raw food, and group 5 feeding on 100 % raw food. The same division were made for dry food that is; group 1 feeding on 0% dry food,

group 2 feeding on 1–30 % dry food, group 3 feeding on 31–60 % dry food, group 4 feeding on 61–99 % dry food, and group 5 feeding on 100 % dry food.

4.2 Blood sampling and analysis

Blood were collected from the vena jugularis into Vacuette® 3ml EDTA and 6 ml plain serum tubes by a closed method (Vacutainer Safety-Lok Blood™ collection sets, Becton, Dickinson, Meylan, France). EDTA samples collected during dog shows were turned around 5 times instantly and then stored in an insulation box until transported to the Helsinki University Veterinary Teaching Hospital. Blood drawn into EDTA tubes from dogs at the hospital were also turned around 5 times instantly and then analysed immediately. Serum samples had to stand for half an hour before centrifuged and analysed. During dog shows serum samples were centrifuged on place and serum was moved into Eppendorf tubes after which they were stored in an insulation box until transported to the Veterinary Teaching Hospital for analysis.

From the EDTA blood samples haematology measurements were determined. The measurements included RBC, HGB, HCT, MCV, MCH, MCHC, thrombocyte count, and WBC. Hematologic analysis were performed by ADVIA 2120i Hematology System with multispecies software (Siemens Healthcare Diagnostics, Tarrytown NY, USA). A cyanmethaemoglobin method was used for haemoglobin.

Biochemical analyses were determined from the plain serum samples and included ALP, ALT, albumin, glucose, phosphate, potassium, sodium, calcium, cholesterol, creatinine, bilirubin, total protein and urea. It was performed using Konelab 30*i* (ThermoFisher Scientific, Vantaa, Finland). All samples were fasting samples.

4.3 Statistical methods

The statistical analysis was performed using IBM SPSS statistics version 24.0.0 for Windows (SPSS Inc. Chicago, IL, USA). Descriptive statistics were performed on the original study population as well as for all the other different diet groups. Tests of normality of the whole study population were performed using the Kolmogorov-Smirnov test. When testing for normality for the different diet groups, the Shapiro-Wilk test was used. For the actual analyses on haematology and serum biochemistry, nonparametric

independent samples test (Kruskal-Wallis) were performed to evaluate the different diet groups. Statistical significance was set at $p < 0.05$.

5 RESULTS

5.1 Study population

Of 111 dogs 107 were included for statistical analysis. One dog was excluded due to cancer whereas three dogs were excluded due to hypothyroidism. Of the 107 dogs included 80 were Staffordshire bull terriers and the rest consisted of 18 different breeds. The breeds rough collie, Continental bulldog, Chihuahua, Dalmatian, flat-coated retriever, Tibetan spaniel, Welsh corgi cardigan, mudi, Australian shepherd, whippet, pitbull mix and Malinois all had one representative. Mixed breed, Leonberger and Berger blanc Suisse had three representatives each whereas the breed Samoyed, German shepherd and American Staffordshire terrier were two each. The age ranged from 0.5 to 13 years with the mean age being 4.38 years. Of the 47 males, 12 were neutered and 35 were intact. Fifteen females were spayed and 45 were intact. Most of the dogs (n= 48) lived in an urban area, 40 dogs lived in a city and 19 dogs in the countryside.

More than half of the population (n= 55) were healthy, 3 had some kind of disease, 43 were atopic and 6 were both atopic and diseased. The 3 dogs with only one disease all had osteoarthritis whereas those that were both diseased and atopic, had either heart disease, otitis, eye disease or osteoarthritis and atopy.

Of the fatty acid supplements 31 dogs ate supplements made of animal fat (type of fat not specified) and 25 vegetable fat. Vitamin supplements were as followed; 11 dogs ate vitamin C, 14 dogs vitamin B, 7 dogs vitamin D and 9 dogs vitamin E. Of these vitamins, 6 dogs received them as a multivitamin. One dog received both C and B vitamin, one dog both B and E vitamin, and one dog C, B, and D vitamin together. Calcium was given to 6 dogs whereas 10 dogs got zinc as a supplement. Joint supplements were given to 12 dogs, *lactobacillus* bacteria to 8 dogs and seaweed to 12 dogs. Magnesium was excluded from the dietary supplements because only one dog received it.

Seven dogs received medications regularly whereas 24 dogs had been given some kind of medication during the last three months. Of these, four dogs received medications both regularly and occasionally during the last three months. One dog received eye drops regularly, two received some kind of NSAID regularly (Trocoxil® [mavacoxib] and Rimadyl® [carprofen]), one ate Rinexin® (phenylpropanolamine), one Antepsin® (sucralfate), one Prednisolon® (prednisolone), and one had Malaseb® (miconazole nitrate and chlorhexidine gluconate) shampooings regularly. One of the NSAID receiving dogs also got Antirobe® (clindamycin) regularly. The medications given during the last three months varied widely. Different antiparasitics had been given to five dogs, Cerenia® (maropitant) to one dog, Canaural® (diethanolamine fusidate, framycetin sulphate, and nystatin) to one dog, sucralfate to one dog, cyclosporine to two dogs, famotidine to one dog, three dogs had been given some kind of antibiotics, 11 dogs NSAIDs, and 4 dogs corticosteroids. One dog had been sedated and anesthetized and had therefore received Propovet Multidose® (propofol), Torpudor Vet® (butorphanol), Dorbene Vet® (medetomidine), and Norocarp Vet® (carprofen). Furthermore, one dog receiving NSAIDs also got tramadol.

Hematologic analyses were performed on only 99 dogs whereas biochemical analyses were performed on all 107 dogs. Two dogs missed the value for total bilirubin. The mean values for haematology and biochemistry were within reference ranges except for MCH and MCHC which were below the reference range (Table 1).

Because of unclear information in the questionnaire, six dogs were excluded from further diet analyses. Five of the six dogs excluded had changed from whole raw food to mixed, and one dog had changed from mixed food to 100% raw food. Therefore, out of 101 dogs, 28 dogs consumed only raw food, 26 only dry food and 47 mixed dry, raw and other foods (canned and homemade food).

Table 1. Mean values of haematology and serum biochemistry of the whole study population (n= 99-107).

	RI	
		Mean±SD
WBC	5.4–17.4x 10^9/l	8.95±2.91
RBC	5.3–8.0x 10^9/l	7.30±0.86
HGB	140–203 g/l	172.42±18.24
HCT	38–57 %	51.51±5.71
MCV	67–80 fl	70.72±3.36
MCH	24–29 pg	23.69±1.11
MCHC	345–367 g/l	335.03±7.82
Thrombocytes	102–395x 10^9/l	345.18±104.38
ALP	33–215 U/I	84.79±53.40
ALT	18–77 U/I	54.09±36.39
Albumin	30–41 g/l	33.28±2.56
Bilirubin	2.5–8.5 µmol/l	2.81±0.91
Phosphate	0.93–2.25 mmol/l	1.20±0.39
Glucose	4.0–6.4 mmol/l	5.49±0.60
Potassium	4.2–5.4 mmol/l	4.39±0.30
Sodium	147–157 mmol/l	148.35±2.16
Calcium	2.3–3.0 mmol/l	2.66±0.12
Cholesterol	3.7–9.8 mmol/l	6.78±1.55
Creatinine	57–116 µmol/l	89.32±12.58
Protein	58–77 g/l	59.50±4.62
Urea	2.4–8.8 mmol/l	6.52±2.40

RI, reference interval. SD, standard deviation. WBC= white blood cell count, RBC= red blood cell count, HGB= haemoglobin, HCT= haematocrit, MCV= mean cell volume, MCH= mean cell haemoglobin, MCHC= mean cell haemoglobin concentration, ALP= alkaline phosphatase, and ALT= alanine aminotransferase.

5.2 Haematology and serum biochemistry

5.2.1 Dogs eating 100% of a specific diet

All measurements were within reference ranges except for MCH and MCHC, which were slightly below the reference range in all diet groups (Table 2 and 3).

Significant differences were found for RBC, HGB and thrombocyte counts between the different diets (Table 2). Further investigation showed that the RBC count differed

between mixed and raw groups (p= 0.007) and between dry and raw groups (p= 0.028). The same was true for the HGB counts (mixed-raw= 0.026 and dry-raw= 0.028). For the thrombocyte count only mixed and raw groups differed significantly (p= 0.001).

The analyses for biochemistry showed statistical difference between diets for ALP, albumin, bilirubin, phosphate, glucose, sodium, cholesterol, creatinine, protein and urea (Table 3). ALP differed between mixed and raw (p= 0.002) as well as dry and raw diets (p= 0.000). For albumin, only the mixed and raw group differed significantly (p= 0.041). While bilirubin showed statistical difference, further investigation did not find any significant differences between the diet groups. Glucose showed statistical difference between mixed and dry groups (p= 0.026). Sodium and phosphate showed differences between mixed and raw (sodium p= 0.000 and phosphate p= 0.003) and mixed and dry (sodium p= 0.001 and phosphate p= 0.007) groups. Cholesterol differed between and mixed and dry (p= 0.020), and dry and raw (p= 0.000) groups. Creatinine differed between the mixed and raw (p= 0.010) and the dry and raw groups (p= 0.001). The protein count only differed between mixed and raw groups (p= 0.016) whereas the urea concentration only differed between mixed and dry groups (p= 0.033).

Table 2. Comparison of mean values on haematology, according to diet fed (sample sizes in parentheses).

	Group	Raw diet (n= 28)	Dry diet (n= 24)	Mixed diet (n= 41)	P-value
	RI	Mean±SD	Mean±SD	Mean±SD	
WBC	5.4–17.4x 10^9/l	8.25±2.17	7.94±2.47	9.87±3.36	0.050
RBC	5.3–8.0x 10^9/l	7.74±0.67	7.11±0.68	7.11±0.98	**0.005**
HGB	140–203 g/l	180.64±15.36	167.71±15.44	169.41 ±20.49	**0.011**
HCT	38–57 %	53.59±4.78	50.36±4.67	50.63±6.64	0.062
MCV	67–80 fl	69.33±3.88	70.91±3.04	71.28±3.11	0.122
MCH	24–29 pg	23.38±1.10	23.62±0.96	23.91±1.21	0.126
MCHC	345–367 g/l	337.32±7.24	333.13±7.85	335.20±8.14	0.133
Thrombocytes	102–395x 10^9/l	389.96±85.15	342.21±109.31	318.07±109.87	**0.002**

In bold are $p < 0.05$. RI, reference interval. SD, standard deviation. The group raw diet consists of dogs feeding on 100 % raw food, dry diet of dogs feeding on 100 % dry food, and mixed diet of dogs that did not fulfil the criteria of either 100 % raw or dry diets. For abbreviations, see table 1.

Table 3. Comparison of mean values on serum biochemistry, according to diet fed (sample sizes in parentheses).

	Group	Raw diet (n= 28)	Dry diet (n= 26)	Mixed diet (n= 47)	P-value
	RI	Mean±SD	Mean±SD	Mean±SD	
ALP	33–215 U/I	52.89±20.22	100.0±46.76	89.34±60.11	**<0.001**
ALT	18–77 U/I	49.25±21.8	58.46±23.78	51.23±39.02	0.113
Albumin	30–41 g/l	34.14±2.49	33.46±1.7	32.7±2.96	**0.041**
Bilirubin*	2.5–8.5 µmol/l	3.1±0.81	3.02±1.15	2.53±0.76	**0.047**
Phosphate	0.93–2.25 mmol/l	1.04±0.29	1.08±0.21	1.35±0.47	**0.001**
Glucose	4.0–6.4 mmol/l	5.44±0.54	5.31±0.43	5.7±0.65	**0.018**
Potassium	4.2–5.4 mmol/l	4.38±0.24	4.33±0.3	4.37±0.30	0.639
Sodium	147–157 mmol/l	149.61±1.59	149.0±1.44	147.17±2.31	**<0.001**
Calcium	2.3–3.0 mmol/l	2.66±0.11	2.66±0.11	2.67±0.14	1.000
Cholesterol	3.7–9.8 mmol/l	6.03±1.10	7.89±1.92	6.60±1.26	**<0.001**
Creatinine	57–116 µmol/l	96.75±13.53	84.85±10.63	88.36±11.73	**0.001**
Protein	58–77 g/l	61.61±6.45	59.77±2.41	58.13±4.07	**0.014**
Urea	2.4–8.8 mmol/l	6.38±1.79	5.86±2.01	7.19±2.83	**0.038**

*Bilirubin, raw n= 27, mixed n= 46. In bold are $p < 0.05$. RI, reference interval. SD, standard deviation. For group description, see table 2. For abbreviations, see table 1.

5.2.2 Staffordshire bull terriers

All measurements were within reference intervals except for MCH, MCHC, thrombocyte, and protein counts. MCH and MCHC counts were below the reference intervals in all diet groups whereas the thrombocyte count was above it in only the raw food group. Protein was slightly below reference intervals in the mixed food group.

Comparison between diet groups of the study population's dominant breed showed similar statistical differences than the comparison of the whole population. Significant differences between diets were shown in RBC, HGB and thrombocyte counts (Table 4). Both RBC (p= 0.018) and HGB (p= 0.013) showed differences between dry and raw groups. The thrombocyte count showed differences between the mixed and raw groups (p= 0.039) and dry and raw groups (p= 0.033).

Of the biochemical parameters, only ALP, glucose, sodium, cholesterol, and creatinine differed significantly (Table 5). ALP and creatinine showed statistical significance

between the mixed and raw groups (ALP= 0.004, creatinine= 0.001) and dry and raw groups (ALP= 0.000, creatinine= 0.006). Glucose and sodium showed statistical significance between the mixed and raw groups (glucose= 0.000, sodium= 0.000) and mixed and dry groups (glucose = 0.000, sodium= 0.001). Cholesterol only showed statistical difference between dry and raw groups (p= 0.000).

When analysing all dogs but Staffordshire bull terriers, the number of dogs in the 100% dry and raw food groups became too small to give statistically reliable results. In the analyses for haematology both the 100% raw and dry diet groups consisted of only two dogs. In the analyses for serum biochemistry the raw diet groups consisted of two dogs whereas the dry diet group consisted of three dogs. Almost all dogs were situated in the mixed diet group (for haematology n= 18 and for serum biochemistry n= 22). Therefore, these analyses were excluded from the study.

Table 4. Comparison of mean values on haematology of only the Staffordshire bull terriers according to diet fed (sample sizes in parentheses).

	Group	Raw diet (n= 26)	Dry diet (n= 22)	Mixed diet (n= 23)	P-value
	RI	Mean±SD	Mean±SD	Mean±SD	
WBC	5.4–17.4x 10^9/l	8.10±2.18	8.12±2.48	9.86±3.76	0.247
RBC	5.3–8.0x 10^9/l	7.77±0.69	7.09±0.65	7.31±1.05	**0.016**
HGB	140–203 g/l	181.0±15.60	166.78±13.97	173.83±21.40	**0.017**
HCT	38–57 %	53.68±4.83	50.08±4.07	52.15±6.87	0.073
MCV	67–80 fl	69.23±3.89	70.69±3.02	71.49±3.45	0.125
MCH	24–29 pg	23.35±1.11	23.54±0.96	23.89±1.42	0.309
MCHC	345–367 g/l	337.46±7.40	333.0±8.02	333.83±8.87	0.061
Thrombocytes	102–395x 10^9/l	401.42±76.0	349.8±109.85	354.13±122.43	**0.013**

In bold are $p < 0.05$. RI, reference interval. SD, standard deviation. For group description, see table 2. For abbreviations, see table 1.

Table 5. Comparison of mean values on serum biochemistry of only the Staffordshire bull terriers, according to diet fed (sample sizes in parentheses).

	Group	Raw diet (n= 26)	Dry diet (n= 23)	Mixed diet (n= 25)	P-value
	RI	Mean±SD	Mean±SD	Mean±SD	
ALP	33–215 U/I	53.31±20.95	104.39±44.56	89.20±57.38	**<0.001**
ALT	18–77 U/I	48.31±22.04	59.35±23.51	57.56±51.90	0.102
Albumin	30–41 g/l	34.30±2.43	33.44±1.74	32.94±3.72	0.128
Bilirubin	2.5–8.5 µmol/l	3.08±0.82	2.98±0.91	2.54±0.58	0.066
Phosphate	0.93–2.25 mmol/l	1.02±0.29	1.05±0.20	1.27±0.55	0.125
Glucose	4.0–6.4 mmol/l	5.41±0.55	5.35±0.40	6.03±0.57	**<0.001**
Potassium	4.2–5.4 mmol/l	4.40±0.23	4.33±0.29	4.43±0.26	0.248
Sodium	147–157 mmol/l	149.85±1.38	149.26±1.29	147.20±2.31	**<0.001**
Calcium	2.3–3.0 mmol/l	2.66±0.01	2.66±0.10	2.67±0.16	0.971
Cholesterol	3.7–9.8 mmol/l	6.06±1.13	8.10±1.85	6.85±1.38	**<0.001**
Creatinine	57–116 µmol/l	94.73±8.43	86.30±10.44	84.8±8.66	**<0.001**
Protein	58–77 g/l	61.81±6.57	60.00±2.41	58.0±4.49	0.056
Urea	2.4–8.8 mmol/l	6.20±1.72	5.84±2.14	6.56±2.36	0.270

In bold are $p < 0.05$. RI, reference interval. SD, standard deviation. For group description, see table 2. For abbreviations, see table 1.

5.2.3 Dogs eating raw; divided into groups

The raw groups were divided into 5 groups to form somewhat even percent intervals. The group division and the percentages of other foods fed in addition to raw food are shown in table 6.

Table 6. Percent intervals of the raw food groups and percentages of additional feedstuff fed. Sample sizes are in parentheses (n= x).

Group	% of raw foods	Mean % of dry foods (min-max)	Mean % of other foods* (min-max)
1 (n= 35)	0 %	90 % (0 % - 100 %)	10 % (0 % - 70 %)
2 (n= 16)	1–30 %	69.66 % (30 % - 90 %)	16.14 % (0 % - 60 %)
3 (n= 16)	31–60 %	42.5 % (0 % - 50 %)	10.42 (0 % - 50 %)
4 (n= 6)	61–99 %	11.67 % (10 % - 20 %)	6.67 % (0 % - 10 %)
5 (n= 28)	100 %	0 %	0 %

*Other foods are canned food and homemade food.

All hematologic and serum biochemical analytes were within reference intervals except for MCH, MCHC and protein. MCH had low counts in groups 1, 4, and 5. The MCHC count were below reference intervals in all groups and protein showed lower counts in group 2 (Table 8 and 9).

Statistical significance was found for WBC, RBC, HGB, HCT, thrombocytes, ALP, phosphate, sodium, cholesterol, creatinine, and protein (Table 8 and 9). The WBC count differed between groups 1 and 2 (p= 0.013), RBC count between groups 2 and 5 (p= 0.001), and HGB count between groups 2 and 5 (p= 0.002). HCT differed significantly between groups 2 and 5(p= 0.005) as well as 2 and 4 (p= 0.049). The thrombocyte count differed between groups 1 and 5 (p= 0.022) and groups 4 and 5 (p= 0.048). ALP differed significantly between three groups; groups 5 and 1 (p= 0.004), groups 5 and 2 (p= 0.013), and groups 5 and 4 (p= 0.043). Phosphate and protein differed both between groups 2 and 5 (phosphate= 0.003, protein= 0.028). Cholesterol showed statistical significance between groups 1 and 5 (p= 0.006). Both creatinine and sodium differed significantly between groups 2 and 5 (p= 0.013 and p= <0.001, respectively). Creatinine also differed between groups 1 and 5 (p= 0.021) whereas sodium also differed between groups 3 and 5 (p= 0.006).

5.2.4 Dogs eating dry; divided into groups

The dry groups were divided into 5 groups to form somewhat even percent intervals. The group division and the percentages of other foods fed in addition to dry food are shown in table 7.

Table 7. Percent intervals of the dry food groups and percentages of additional feedstuff fed. Sample sizes are in parentheses (n= x).

Group	% of dry foods	Mean % of raw foods (min-max)	Mean % of other foods* (min-max)
1 (n= 29)	0 %	98.28 % (50 % - 100 %)	1.72 % (0 % - 50 %)
2 (n= 13)	1–30 %	45.38 % (0 % - 90 %)	33.08 % (0 % - 70 %)
3 (n= 18)	31–60 %	37.41 % (0 % - 60 %)	13.7 (0 % - 50 %)
4 (n= 15)	61–99 %	10.48 % (0 % - 30 %)	5.88 % (0 % - 20 %)
5 (n= 26)	100 %	0 %	0 %

*Other foods are canned food and homemade food.

All hematologic and serum biochemical analytes were within reference intervals except for MCH, MCHC, thrombocyte, bilirubin, and protein counts. MCH and MCHC showed lower counts than the reference interval in all groups. Bilirubin in group 4 and protein in group 3 showed lower counts than the reference interval. The thrombocyte count were above reference intervals in group 1 (Table 10 and 11).

Statistical significance was found for RBC, HGB, thrombocytes, ALP, ALT, phosphate, glucose, sodium, cholesterol, creatinine, and protein (Table 10 and 11). For RBC, HGB, ALT, and glucose further investigations showed no significant statistical difference between the groups. Sodium differed statistically between the following groups; groups 1 and 2 ($p= 0.002$), 1 and 3 ($p= 0.007$), 1 and 4 ($p= 0.006$), and 2 and 5 ($p= 0.035$). The thrombocyte count differed between groups 1 and 2 ($p= 0.001$), creatinine between groups 1 and 5 ($p= 0.002$), protein between groups 1 and 3 ($p= 0.034$), cholesterol between groups 1 and 5 ($p= 0.002$), and phosphate between groups 1 and 4 ($p= 0.043$). ALP differed significantly between groups 1 and 5 ($p= 0.001$).

To better visualize the equivalence of the raw food groups and the dry food groups, mean values for every respective group were visualized in diagrams. This was made for all hematologic and serum biochemical analytes but only RBC, HGB, total protein, creatinine, cholesterol, sodium, and glucose are shown here (Fig. 1–7).

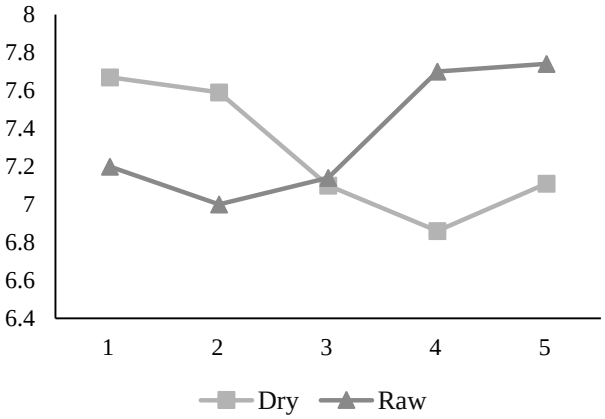

Fig 1. Mean erythrocyte concentrations according to the raw and dry food groups. Group one is 0 % raw/dry food, group 2 is 1–30 % raw/dry food, group 3 is 31–60 % raw/dry food, groups 4 is 61–99 % raw/dry food, and group 5 is 100 % raw/dry food

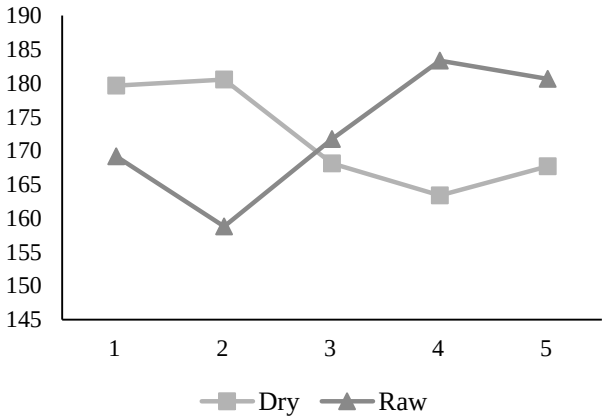

Fig 2. Mean haemoglobin concentrations according to the raw and the dry food groups. For group description, see fig. 1.

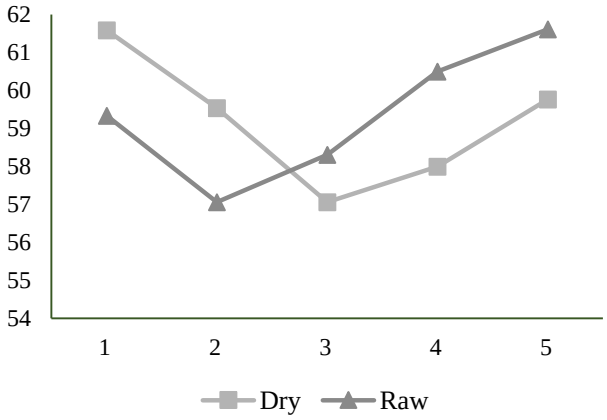

Fig 3. Mean total protein concentrations according to the raw and dry food groups. For group description, see fig 1.

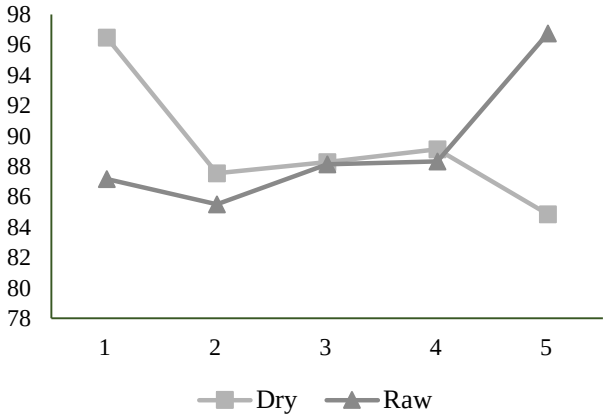

Fig 4. Mean creatinine concentrations according to the raw and dry food groups. For group description, see fig 1.

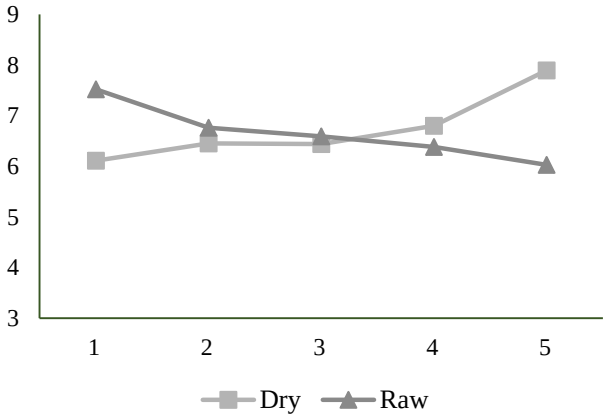

Fig 5. Mean cholesterol concentrations according to the raw and dry food groups. For group description, see fig 1.

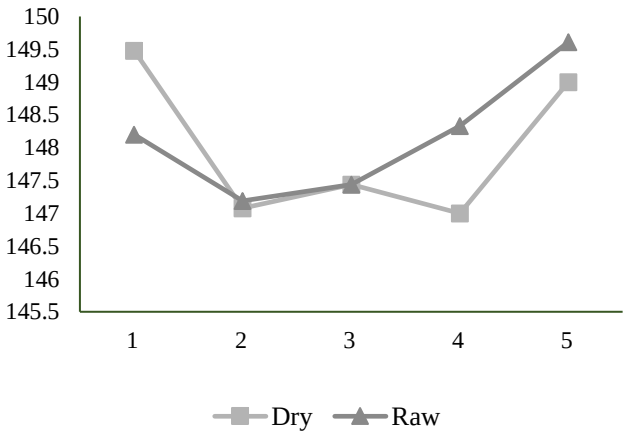

Fig 6. Mean sodium concentrations according to the raw and dry food groups. For group description, see fig 1.

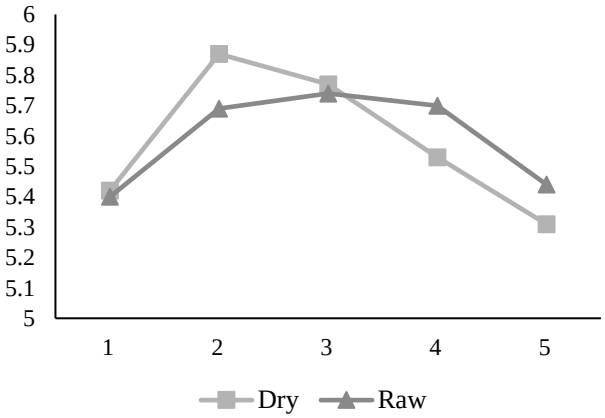

Fig 7. Mean glucose concentrations according to the raw and dry groups. For group abbreviations, see fig 1.

Table 8. Comparison of mean values on haematology of the dogs in raw food groups 1–5 (sample sizes in parentheses).

	Group	1 (n= 31)		2 (n= 14)		3 (n= 14)		4 (n= 6)		5 (n= 28)		P-value
	RI	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
WBC	5.4–17.4x 10^9/l	7.88	±2.36	10.85	±2.44	9.76	±4.34	10.41	±3.35	8.25	±2.17	**0.011**
RBC	5.3–8.0x 10^9/l	7.20	±0.71	6.63	±0.97	7.14	±0.98	7.70	±0.91	7.74	±0.67	**0.001**
HGB	140–203 g/l	169.16	±15.10	158.79	±22.27	171.71	±18.0	183.33	±20.06	180.64	±15.36	**0.002**
HCT	38–57 %	50.67	±4.65	47.40	±6.65	51.31	±6.46	55.28	±6.67	53.59	±4.78	**0.006**
MCV	67–80 fl	70.47	±3.32	71.56	±2.52	71.95	±3.42	71.77	±1.30	69.33	±3.88	0.146
MCH	24–29 pg	23.54	±1.06	23.99	±0.91	24.16	±1.53	23.87	±0.52	23.38	±1.10	0.115
MCHC	345–367 g/l	333.94	±7.63	335.29	±5.40	335.43	±11.37	332.67	±7.37	337.32	±7.24	0.409
Thrombocytes	102–395x 10^9/l	324.87	±107.18	328.50	±54.02	347.57	±166.23	286.33	±41.76	389.96	±85.15	**0.008**

In bold are $p < 0.05$. RI, reference interval. SD, standard deviation. Group 1 is 0 % raw food, group 2 is 1-30 % raw food, group 3 is 31-60 % raw food, group 4 is 61-99 % raw food, and group 4 is 100 % raw food. For abbreviations, see table 1.

Table 9. Comparison of mean values on serum biochemistry of the dogs in raw food groups 1–5 (sample sizes in parentheses).

	Group	1 (n= 35)		2 (n=16)		3 (n= 16)		4 (n= 6)		5 (n= 28)		P-value
	RI	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
ALP	33–215 U/I	89.06	±46.75	114.50	±86.82	79.44	±34.01	96.50	±37.65	52.89	±20.22	**0.001**
ALT	18–77 U/I	53.66	±22.53	47.06	±20.47	60.50	±63.18	54.83	±5.91	49.25	±21.80	0.368
Albumin	30–41 g/l	33.20	±1.93	32.68	±2.74	32.49	±3.56	33.73	±3.02	34.14	±2.49	0.246
Bilirubin*	2.5–8.5 µmol/l	2.89	±1.09	2.25	±0.79	2.70	±0.98	2.80	±0.98	3.10	±0.81	0.124
Phosphate	0.93–2.25 mmol/l	1.15	±0.25	1.56	±0.61	1.17	±0.39	1.23	±0.36	1.04	±0.29	**0.008**
Glucose	4.0–6.4 mmol/l	5.40	±0.51	5.69	±0.80	5.74	±0.52	5.70	±0.68	5.44	±0.54	0.163
Potassium	4.2–5.4 mmol/l	4.31	±0.28	4.44	±0.29	4.39	±0.29	4.33	±0.48	4.38	±0.24	0.519
Sodium	147–157 mmol/l	148.20	±2.49	147.19	±1.80	147.44	±2.22	148.33	±0.82	149.61	±1.59	**<0.001**
Calcium	2.3–3.0 mmol/l	2.65	±0.12	2.69	±0.12	2.66	±0.17	2.70	±0.16	2.66	±0.11	0.929
Cholesterol	3.7–9.8 mmol/l	7.52	±1.79	6.76	±1.52	6.59	±1.27	6.38	±1.40	6.03	±1.10	**0.013**
Creatinine	57–116 µmol/l	87.17	±10.42	85.50	±11.76	88.13	±11.22	88.33	±18.10	96.75	±13.53	**0.007**
Protein	58–77 g/l	59.34	±2.55	57.06	±4.28	58.31	±4.56	60.50	±3.56	61.61	±6.45	**0.035**
Urea	2.4–8.8 mmol/l	6.50	±2.35	6.86	±3.10	7.02	±2.42	6.78	±3.93	6.38	±1.79	0.866

In bold are p < 0.05. RI, reference interval. SD, standard deviation. For group description, see table 8. For abbreviations, see table 1.
*Bilirubin: group 2 n= 15, group 5 n= 27.

Table 10. Comparison of mean values on haematology of the dogs in dry food groups 1–5 (sample sizes in parentheses).

	Group	1(n= 29)		2 (n= 11)		3 (n= 15)		4 (n= 14)		5 (n= 24)		P-value
	RI	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
WBC	5.4–17.4x 10^9/l	8.13	±2.22	9.55	±3.30	10.73	±4.04	9.55	±2.45	7.94	±2.47	0.093
RBC	5.3–8.0x 10^9/l	7.67	±0.76	7.59	±0.98	7.10	±0.92	6.86	±0.96	7.11	±0.68	**0.019**
HGB	140–203 g/l	179.62	±16.06	180.55	±22.54	168.13	±18.74	163.36	±18.70	167.71	±15.44	**0.027**
HCT	38–57 %	53.26	±5.23	54.05	±7.45	50.49	±6.03	48.77	±5.79	50.36	±4.67	0.086
MCV	67–80 fl	69.43	±3.85	71.17	±1.83	71.26	±3.71	71.31	±3.51	70.91	±3.04	0.454
MCH	24–29 pg	23.49	±1.22	23.83	±0.45	23.78	±1.48	23.92	±1.21	23.62	±0.96	0.492
MCHC	345–367 g/l	338.31	±8.88	334.81	±7.65	333.27	±7.06	335.36	±5.26	333.13	±7.85	0.233
Thrombocytes	102–395x 10^9/l	405.03	±116.52	268.45	±72.31	318.73	±88.39	320.00	±51.93	342.21	±109.31	**0.001**

In bold are $p < 0.05$. RI, reference interval. SD, standard deviation. Group 1 is 0 % dry food, group 2 is 1-30 % dry food, group 3 is 31-60 % dry food, group 4 is 61-99 % dry food, and group 4 is 100 % dry food For abbreviations, see table 1.

Table 11. Comparison of mean values on serum biochemistry of dogs in dry food groups 1–5 (sample sizes in parentheses).

	Group	1 (n= 29)		2 (n= 13)		3 (n= 18)		4 (n= 15)		5 (n= 26)		P-value
	RI	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
ALP	33–215 U/I	55.17	±23.34	79.46	±38.33	102.61	±82.87	80.00	±42.42	100.00	±46.76	**0.001**
ALT	18–77 U/I	48.86	±21.51	73.23	±69.26	44.56	±11.71	41.07	±9.70	58.46	±23.78	**0.045**
Albumin	30–41 g/l	34.04	±2.50	32.75	±3.39	33.10	±3.42	32.27	±2.06	33.46	±1.70	0.127
Bilirubin*	2.5–8.5 µmol/l	3.07	±0.81	2.75	±0.87	2.54	±0.70	2.33	±0.76	3.02	±1.15	0.131
Phosphate	0.93–2.25 mmol/l	1.05	±0.29	1.23	±0.38	1.43	±0.65	1.36	±0.29	1.08	±0.21	**0.006**
Glucose	4.0–6.4 mmol/l	5.42	±0.54	5.87	±0.77	5.77	±0.54	5.53	±0.65	5.31	±0.43	**0.020**
Potassium	4.2–5.4 mmol/l	4.39	±0.26	4.39	±0.37	4.29	±0.21	4.41	±0.31	4.33	±0.30	0.572
Sodium	147–157 mmol/l	149.48	±1.70	147.08	±1.75	147.44	±2.12	147.00	±3.02	149.00	±1.44	**<0.001**
Calcium	2.3–3.0 mmol/l	2.67	±0.13	2.64	±0.15	2.70	±0.13	2.64	±0.10	2.66	±0.11	0.520
Cholesterol	3.7–9.8 mmol/l	6.11	±1.16	6.45	±1.43	6.44	±1.13	6.80	±1.28	7.89	±1.92	**0.004**
Creatinine	57–116 µmol/l	96.48	±13.36	87.54	±14.55	88.28	±11.07	89.13	±10.97	84.85	±10.63	**0.005**
Protein	58–77 g/l	61.59	±6.34	59.54	±3.43	57.06	±4.98	58.00	±3.21	59.77	±2.41	**0.021**
Urea	2.4–8.8 mmol/l	6.40	±1.76	6.98	±2.97	7.33	±2.69	7.22	±3.14	5.86	±2.01	0.159

In bold are $p < 0.05$. RI, reference interval. SD, standard deviation. For group description, see table 10. For abbreviation, see table 1.
*Bilirubin: group 1 n = 28, group 4 n= 14.

6 DISCUSSION

In our present study we report significant differences in both haematological and serum biochemical blood parameters between different diets. In the whole study population most differences were found between the mixed and raw diets (RBC, HGB, thrombocyte count, ALP, albumin, sodium, phosphate, creatinine, and protein), whereas in the group of only Staffordshire bull terriers, differences between the raw and dry diets were found for RBC, HGB, thrombocyte count, ALP, creatinine, and cholesterol.

For the five different raw diet groups, divided by percentage of raw food, 7 parameters were shown to differ significantly between groups 2 (raw food 1-30 %, mean percent of dry food 69.66 %, and mean percent of other foods 16.14 %) and 5 (raw food 100 %). These parameters were RBC, HGB, HCT, ALP, phosphate, protein, creatinine, and sodium. Between groups 1 (raw food 0 %, mean percent of dry food 90 %, and mean percent of other foods 10 %) and 5, thrombocyte count, ALP, creatinine, and cholesterol differed significantly. For the five different dry food groups, divided by percentage of dry food, most parameters differed significantly between groups 1 (dry food 0 %, mean percent of raw food 98,28 %, and mean percent of other foods 1.72 %) and 5 (dry food 100 %). These were creatinine, ALP, and cholesterol. Both these results are, as expected, in line with the above mentioned results of the 100 % raw diet groups since they really are each other's opposites.

Common to both the whole population and Staffordshire bull terriers were differences between the raw and dry diets for RBC, HGB, ALP, creatinine, and cholesterol. Mixed and raw diets differed significantly for thrombocyte count, ALP, sodium, and creatinine. Only sodium differed significantly between the dry and mixed diets, for both populations. Most of the parameters followed the same trend (increased or decreased values) in both analyses. RBC and HGB were highest in the raw diet and lowest when consuming a dry diet. Thrombocyte count were highest in the raw diet but lowest in the mixed diet when analysing the whole study population, respectively in the dry diet when analysing only the Staffordshire bullterriers. ALP and cholesterol where highest in the dry diet and lowest in the raw diet for both study populations. Sodium showed high values in the raw diet and low values in the mixed diet. Creatinine also had the highest concentrations in

the raw diet and lowest in the dry diet (for the whole population) and mixed diet (Staffordshire bull terriers).

For both study groups MCH and MCHC were slightly below reference ranges. Since the majority of the study population consisted of Staffordshire bullterriers, this could be due to breed-specific variations. Both blood parameters have been found to vary between breeds (Nielsen et al. 2010, Lavoué et al. 2014), indicating that this could also be the case for Staffordshire bull terriers. Individual variations may also affect the mean values to some extent. The thrombocyte counts were above reference ranges in the raw diet group of only Staffordshire bull terriers. Again, this could indicate breed-specific variations, but since it was only elevated for the dogs consuming a raw diet, a more likely cause would be that the diet affected the thrombocyte count. The same can also be seen in the analysis of the different dry food percentages where thrombocyte count is above reference ranges in group 1 (0 % dry food, 98.28 % raw food, and 1.72 % other food), further supporting the effect of diet on the thrombocyte count. This needs to be further evaluated since no studies were found to report variations in thrombocyte counts according to different diets.

The RBC count was highest when feeding 100 % raw food or 0 % dry food. The RBC counts were the same in both raw and dry diet groups when feeding 31–60 % of either one and when feeding 0 % raw food. In these groups both raw and dry food diets had similar percentages of other foods (raw food group 3: 10 %, dry food group 3: 14 %, and raw food group 1: 10 %). Raw food usually contains a lot of raw meat which naturally increases the RBC but why does the three different diet groups show the same RBC concentrations? A possible explanation could be that people feeding mostly dry food will complement the diet with more protein (minced meat, fish, chicken etc.) whereas those feeding a more equal distribution of raw and dry food complements the diet with carbohydrates (cooked rice, potato mash, carrots etc.). Since this study didn't specify this, any conclusions about the food items are hard to make. Further studies are needed to find out dog owners' exact feeding regimen and whether it affects blood parameters or not.

In summary, the haematological values increased when the amount of raw food increased in the diet, except for MCV and MCH. The most common groups where blood parameters were the same, were groups 3 in both raw diet and dry diet (31-60 %). This was because the composition of raw, dry and other foods was similar in this group and small changes would probably not affect the values.

Creatinine, ALP, and cholesterol showed strong difference between dry and raw food diets. This can especially be seen for creatinine and cholesterol which nearly form opposite linear curves (Fig. 4 and fig. 5). Creatinine increased when the amount of raw food increased and respectively decreased with higher amounts of dry food. However, a prior study showed that processed food had higher amounts of creatinine compared to raw food, which again had higher creatine concentrations (Dobenecker and Braun 2015). Why is it then that dogs consuming raw food have higher creatinine concentrations than dogs consuming a dry food diet? This could be because of effective secretion of creatinine through the kidneys and absorption of creatine to the muscle. Ingested creatinine will not persist long in the blood plasma whereas excessive creatine in the muscle will slowly degrade into creatinine leading to higher concentrations. Muscle mass affects the creatinine output in plasma and therefore more creatine will lead to more creatinine in the blood (Braun et al. 2003). Another important aspect with the increased creatinine values in the raw fed dogs is that increased creatinine is normally used as a marker for abnormal kidney function (Braun et al. 2003). Therefore, there is a possibility that dogs eating a raw food diet could be misdiagnosed if the clinician uses normal reference intervals and does not take into account the possible effects of the dog's diet.

In our study, we showed that cholesterol decreased with increased amounts of raw food and increased with increased amounts of dry food. Since raw food usually has a high fat and high protein content (Buff et al. 2014, review) our results are in contrast with Kronfeld et al. (1977), who showed that increased fat and protein in the diet would lead to increased, instead of decreased, cholesterol concentrations. However, more recent findings by de Godoy et al. (2014) shows decreased cholesterol concentrations when feeding a high protein and fat diet, thus strengthening the results seen in our study. A diet rich in fish and fish by-products has also been found to decrease cholesterol concentrations (Pasquini et al. 2008). Fish is usually fat and a source of cholesterol but contains less saturated fat, indicating that dietary cholesterol might not be the one affecting blood cholesterol concentrations (Kanter et al. 2012). Our findings could also be of special importance to human medicine, were cholesterol and its connection to many diseases has been extensively studied. Since it clearly shows that diet has an impact on cholesterol concentrations in dogs, it could give clues for further studies in humans.

The total protein concentration was slightly below reference ranges in the raw food group 2 (1-30 %) and dry food group 3 (31-60 %) indicating a possible protein insufficiency

when mixing diets. In raw food group 2 the amount of dry food was 70 % and the amount of other foods 16 % leading to a raw food amount of only 14 %. In dry food group 3 the raw food and other foods stood for 51 % (raw food 37 %, other foods 14 %) of the diet whereas dry food stood for 49 % of the diet. Since we don't know what exactly the owners have been feeding to the dogs, the other foods might consist of e.g. mostly carbohydrate-rich food scraps and raw food could be meaty food scraps (minced meat, bones etc.). Commercial dry food is also only complete if feeding the amounts recommended on the package (Chandler and Takashima 2014, review), therefore a deviation from the recommendations could lead to insufficient protein intake. If not complementing with other feedstuff, it could lead to decreased protein concentrations as seen in our results. The protein curve seen in our study (fig. 3) further confirms the thought that commercial dry foods have adequate protein if the dog is fed enough, i.e. according to the package instructions. Both curves increase when the percentage of either raw or dry food increases, although raw food diets naturally gives the highest concentrations. However, the protein concentration in our study was only slightly decreased and would not be interpreted as abnormal.

Albumin also shows similar curves as total protein, which is expected since one component of total protein is albumin. Albumin shows high concentrations when feeding 100 % raw and 0 % dry food and low concentrations when feeding on 31-60 % raw food and 61-99 % dry food. Interestingly 0 % raw food gives higher concentrations than most dry food dominated combinations. This further indicates that commercial dry foods have sufficient amounts of nutrients only when eating it according to package recommendations.

The sodium concentration curve shows an increase with increased amounts of raw food. Also increased amounts of dry food increases the sodium concentration, but why is it that raw food gives higher values than dry food? It might be due to owners with good knowledge of raw food diets that understands to add salt to the diet. The salt amount in commercial dry food diets are usually measured to be sufficient when feeding the recommended amount. Therefore, combinations of raw, dry and other foods could give lower concentrations than complete raw or dry diets. The lowest concentrations were given when the amount of other foods was the highest, indicating that home-made food possibly lacks salt. Foods that have low sodium content include bread, cereal, potatoes, and vegetables (In the book by Hand et al. 2010), and these might be the food items most

often added to mixed diets. Also, salt is often considered a bad thing for humans and this might be reflected to dogs when given home-made food or food scraps (e.g. owners will not season the food with salt).

The glucose concentration curve is lowest when feeding either raw or dry food. Raw food is naturally low in glucose and commercial dry food diets of good quality is also low in glucose. In contrast, a combination of dry, raw and other foods, where the amount of other foods is high, gives higher glucose concentrations. This shows the value of the "other foods" group because it is the one increasing the concentration. What do people feed their dogs since the concentration increases? A possibility is that those owners feeding their dogs a combination of diets are not as educated as e.g. raw feeders, and therefore gives mostly food scraps and other feedstuffs that can be found from the refrigerator (e.g. minced meat, cooked rice, pasta or potatoes, some cooked vegetables, bread, and wheat buns). Food scraps and especially carbohydrate-rich items are probably the ones increasing the glucose concentration.

Overall, a combination of either 31-60 % raw or 31-60 % dry food will give mean concentrations of most of the serum biochemical parameters (phosphate, bilirubin, glucose, sodium, creatinine, and cholesterol). A complete and balanced diet is enough to meet sufficient nutrient concentrations, but too much mixing of diets will lead to lower concentrations, perhaps even insufficient concentrations. The group "other foods" stands for a wide range of different feedstuffs and homemade food is probably the one that varies the most in content. This we cannot know for sure since the questionnaire didn't specify details about the diets.

Because most of the measured blood values stayed within reference ranges the clinical importance and need for specific reference intervals are yet to be determined. It is also good to highlight that the reference intervals used in this study were made from a population of normal dogs eating mostly dry food. Therefore, the need for diet-specific reference intervals cannot be excluded, since some parameters did show variations due to diet.

Since the study population mainly consisted out of one breed (Staffordshire bull terriers, n= 80) and a lot of other breeds with few representatives, the results cannot be interpreted on dogs in general. The bias of breed-specific differences might influence the results and can therefore only give directions on the effects of diet on haematology and serum

biochemistry. Further studies should include a wider, and more even, distribution of different breeds so that the study population would represent dogs in general.

Another limitation of the study would be the inclusion of animals receiving medication and unhealthy animals. Individual variations are known to affect study results (Choi et al. 2011), and therefore the optimal individual would be a completely healthy animal. The exclusion of dogs with cancer and hypothyroidism aimed to minimize possible effects of disease on blood parameters but dogs with e.g. osteoarthritis, atopy, and heart disease were included. These diseases are probably not systemic to the same extent as cancer and hypothyroidism, but it cannot be excluded as a possible limitation of this study. Among the medications given regularly, at least glucocorticoids are known to affect some blood values and have no effect on others (Ginel et al. 2002, Kovalik et al. 2012). Since it was not specified exactly when the medications were given during the last three months in this study, we cannot know for sure whether the medication has affected the blood parameters at the time of analyse, or not. Also, only one dog received glucocorticoids regularly and therefore, should not affect the results.

Also, variables such as age and sex were not taken into account in this study further indicating possible biases of the results. Sex and neutering status has been shown to affect blood parameters, as well as age (Lawrence et al. 2013, Rørtveit et al. 2015). However, regarding age, most differences has been shown to occur at a very young age (Harper et al. 2003). With the mean age being about 5 years in this study, most values would be expected to be within normal reference ranges.

Lastly, the questionnaire did not specify what raw food is and did not ask for what kind of feedstuffs that is given (e.g. what type of meat, vegetables, organs etc.). This is a limitation because when an owner has stated the diet to be 100 % raw food it doesn't give us any information about what kind of raw food it is. Not all people know the true definition of raw food and could have misunderstood for example cooked meat to be raw food. We also do not know what kind of dry food or other foods the owners give the dogs and can therefore only speculate about the meaning of the results and what different owners give to their dogs. The amount of other foods was fairly high in groups 3 of both dry and raw food, and in further studies it would be of importance to let the owner specify what kind of other foods that are given so that more accurate analyses can be made.

7 CONCLUSIONS

To really investigate the true effects of different diets on blood parameter, further studies with bigger study populations and more even spectrum of breeds, are needed. Also, more specific information on the diet, preferably a diet diary of 5-7 days, is required to better interpret the results and understand what elements are affecting the most. This study gave evidence that diet is affecting blood parameters but to which extent, remains unclear. Also, the effects of other parameters such as age, gender, disease etc. remains to be investigated more thoroughly. Since nearly all measured values, except MCH, MCHC, total protein, and thrombocyte count, were within reference intervals, we cannot conclude whether the differences are big enough to be taken into account in clinical diagnostics. But they might help us when we are looking into possible disease pathophysiologies.

8 ACKNOWLEDGEMENTS

I would like to thank my instructor Johanna Anturaniemi for the major help and good advice given during this process. I would also like to thank my director Anna Hielm-Björkman for her never ending enthusiasm on this subject. Last but not least a big thank you goes to all participants in the Tutkielmatyöpaja who has contributed with valuable questions, ideas and support during the start of this thesis.

APPENDIX I

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI

Verinäytelomake - DOGRISK-tutkimus

Täyttäkää tämä lomake huolellisesti ennen kuin tuotte koiranne verinäytteenottoon. Näin toiminta näytteenottopaikalla nopeutuu teidän ja koiranne osalta. Teidän tarvitsee vain täyttää ja lähettää lomake, niin liitämme vastauslomakkeen tiedot ja verinäytteet yhteen paikan päällä. Voitte täyttää lomakkeen siitäkin huolimatta, vaikka ette olisi vielä varma tulostanne verinäytteenottoon. Lomakkeen onnistuneen lähettämisen jälkeen teidät ohjataan automaattisesti tutkimusryhmämme kotisivuille. Kiitos osallistumisestanne!

PERUSTIEDOT

* Koiran kutsumanimi
* Koiran rotu
* Koiran syntymäaika
* Koiran virallinen nimi ja rek.nro
* Omistajan nimi
* Omistajan sähköpostiosoite
* Koiran sukupuoli --Valitse tästä--
* Koira asuu pääasiassa --Valitse tästä--

KOIRAN TERVEYDENTILA

Koiran sairaudet

- Iho-oireita aiheuttava atopia/allergia
- Suolisto-oireita aiheuttava allergia
- Nivelrikko
- Kilpirauhasen vajaatoiminta
- Epilepsia
- Sokeritauti
- Toistuvia korvatulehduksia
- Demodikoosi
- Furunkuloosia
- Syöpä
- Toistuvia virtsatietulehduksia
- Munuaissairaus
- Maksasairaus
- Sydänsairaus
- Spondyloosi
- Osteokondrosis dissecans
- Cushingin tauti
- Addisonin tauti
- Silmäsairaus
- Haiman vajaatoiminta
- AIHA

Jokin muu sairaus, mikä?

Luetelkaa koiranne käyttämät säännölliset lääkitykset tähän

Kirjoittakaa tähän myös koiranne käyttämät satunnaiset lääkkeet viimeisen kuukauden ajalta, sillä ne saattavat vaikuttaa verinäytetuloksiin

KOIRAN RUOKINTA

Koiran ruokavalio viimeisen kolmen kuukauden ajan ?

	0 %	10 %	20 %	30 %	40 %	50 %	60 %	70 %	80 %	90 %	100 %
* Kuivamuonaa											
* Raakaruokaa											
* Kypsää kotiruokaa											
* Koiranmakkaraa ja säilykeruokia											

Jos olette muuttaneet koiranne ruokavaliota viimeisen kolmen kuukauden aikana, merkitkää alle, miten koira aikaisemmin söi ?

	0 %	10 %	20 %	30 %	40 %	50 %	60 %	70 %	80 %	90 %	100 %
Kuivamuonaa											

Raakaruokaa	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Kypsää kotiruokaa	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
Koiranmakkaraa ja säilykeruokia	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

Säännöllisesti käytetyt lisäravinteet

- ☐ Eläinperäiset rasvahapot
- ☐ Kasviperäiset rasvahapot
- ☐ Monivitamiini
- ☐ Kalkki
- ☐ Maitohappobakteerit
- ☐ Merilevävalmiste
- ☐ Biotiini/B-vitamiini
- ☐ Glukosamiini/kondroitiinisulfaatti
- ☐ Magnesium
- ☐ Sinkki
- ☐ C-vitamiini

Jokin muu lisäravinne, mikä?

Lisäksi koira syö säännöllisesti

	Kyllä	Ei
* Nahkaluita	☐	☐
* Aitoja luita	☐	☐
* Makupaloja	☐	☐
* Maitotuotteita	☐	☐
* Eläinten ulosteita	☐	☐

KOIRAN LIIKUNTA

Koiran lenkkeily ilman harrastuksia

	alle tunnin	1-2 tuntia	yli kaksi tuntia
* Koira lenkkeilee arkisin päivässä	☐	☐	☐
* Koira lenkkeilee viikonloppuisin päivässä	☐	☐	☐

Koiran lenkit ovat luonteeltaan

- ☐ Koira on aina hihnassa
- ☐ Koira on välillä hihnassa ja välillä vapaana
- ☐ Koira on aina vapaana
- ☐ Koiralla on lisäksi oma piha, missä se on vapaana
- ☐ Koiran lenkkimaasto on lähinnä tasaista tietä
- ☐ Koiran lenkkimaasto on vaihtelevaa maastoa

Koiran kanssa harrastetaan VIIKOITTAIN

- ☐ Agility
- ☐ TOKO
- ☐ Koiratanssi
- ☐ Jälki
- ☐ Esine-etsintä
- ☐ Rally-TOKO
- ☐ Perus-/arkitottis
- ☐ Taakanveto
- ☐ DOBO
- ☐ Näyttelyt

Muuta, mitä?

Koiran kanssa harrastetaan SATUNNAISESTI

- ☐ Agility
- ☐ TOKO
- ☐ Koiratanssi
- ☐ Jälki
- ☐ Esine-etsintä
- ☐ Rally-TOKO
- ☐ Perus-/arkitottis
- ☐ Taakanveto
- ☐ DOBO
- ☐ Näyttelyt

Muuta, mitä?

KOIRAN SUKULAISTIEDOT

Emän sairaudet

Isän sairaudet

Täyssisarusten sairaudet

Puolisisarusten sairaudet (eri emä/isä)

Pentujen sairaudet

TIETOJEN LÄHETYS

Tallenna

REFERENCES

Arendt M, Fall T, Lindblad-Toh K, Axelsson E. Amylase activity is associated with AMY2B copy numbers in dog: Implications for dog domestication, diet and diabetes. Anim Genet 2014, 45: 716–722.

Axelsson E, Ratnakumar A, Arendt ML, Maqbool K, Webster MT, Perloski M, Liberg O, Arnemo JM, Hedhammar A, Lindblad-Toh K. The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature 2013, 495: 360–364.

Beloshapka AN, Duclos LM, Vester Boler BM, Swanson KS. Effects of inulin or yeast cell-wall extract on nutrient digestibility, fecal fermentative end-product concentrations, and blood metabolite concentrations in adult dogs fed raw meat-based diets. Am J Vet Res 2012, 73: 1016–1023.

Bosch G, Hagen-Plantinga EA, Hendriks WH. Dietary nutrient profiles of wild wolves: insights for optimal dog nutrition? Br J Nutr 2015, 113 Suppl: S40–S54.

Braun JP, Lefebvre HP, Watson AD. Creatinine in the dog: a review. Vet Clin Pathol 2003, 32: 162–179.

Buff PR, Carter RA, Bauer JE, Kersey JH. Natural pet food: a review of natural diets and their impact on canine and feline physiology. J Anim Sci 2014, 92: 3781–3791.

Chandler ML, Takashima G. Nutritional Concepts for the Veterinary Practitioner. Vet Clin N Am: Small Anim Pract 2014, 44: 645–666.

Chang YM, Hadox E, Szladovits B, Garden OA. Serum Biochemical Phenotypes in the Domestic Dog. PLoS One 2016, 11: e0149650.

Choi SY, Hwang JS, Kim IH, Hwang DY, Kang HG. Basic data on the hematology, serum biochemistry, urology, and organ weights of beagle dogs. Lab Anim Res 2011, 27: 283–291.

Davenport DJ, Mostardi RA, Richardson DC, Gross KL, Greene KA, Blair K. Protein-deficient diet alters serum alkaline phosphatase, bile acids, proteins and urea nitrogen in dogs. J Nutr 1994, 124: 2677S–2679S.

de Godoy MR, Beloshapka AN, Carter RA, Fascetti AJ, Yu Z, McIntosh BJ, Swanson KS, Buff PR. Acute changes in blood metabolites and amino acid profile post-exercise in Foxhound dogs fed a high endurance formula. J Nutr Sci 2014, 3: e33.

Diez M, Michaux C, Jeusette I, Baldwin P, Istasse L, Biourge V. Evolution of blood parameters during weight loss in experimental obese Beagle dogs. J Anim Physiol Anim Nutr (Berl) 2004, 88: 166–171.

Dobenecker B, Braun U. Creatine and creatinine contents in different diet types for dogs - effects of source and processing. J Anim Physiol Anim Nutr (Berl) 2015, 99: 1017–1024.

Elliott KF, Rand JS, Fleeman LM, Morton JM, Litster AL, Biourge VC, Markwell PJ. A diet lower in digestible carbohydrate results in lower postprandial glucose concentrations compared with a traditional canine diabetes diet and an adult maintenance diet in healthy dogs. Res Vet Sci 2012, 93: 288–295.

Evira. Salmonella. https://www.evira.fi/sv/djur/djurhalsa-och-sjukdomar/djursjukdomar/gemensamma-sjukdomar-for-flera-arter/salmonella/, received 11.01.2017, last edited 15.11.2016.

Ginel PJ, Lucena R, Fernández M. Duration of increased serum alkaline phosphatase activity in dogs receiving different glucocorticoid doses. Res Vet Sci. 2002; 72: 201–4.

Fieten H, Biourge VC, Watson AL, Leegwater PAJ, van den Ingh TSGAM, Rothuizen J. Nutritional management of inherited copper-associated hepatitis in the Labrador retriever. The Veterinary Journal 2014, 199: 429–433.

Finley R, Ribble C, Aramini J, Vandermeer M, Popa M, Litman M, Reid-Smith R. The risk of salmonellae shedding by dogs fed *Salmonella*-contaminated commercial raw food diets. Canadian Veterinary Journal-Revue Veterinaire Canadienne 2007, 48: 69–75.

Frassetto LA, Schloetter M, Mietus-Synder M, Morris RC,Jr, Sebastian A. Metabolic and physiologic improvements from consuming a paleolithic, hunter-gatherer type diet. Eur J Clin Nutr 2009, 63: 947–955.

Fredriksson-Ahomaa M, Heikkila T, Pernu N, Kovanen S, Hielm-Bjorkman A, Kivisto R. Raw Meat-Based Diets in Dogs and Cats. Vet Sci 2017, 4: 10.3390/vetsci4030033.

Freedman AH, Gronau I, Schweizer RM, Ortega-Del Vecchyo D, Han E, Silva PM, Galaverni M, Fan Z, Marx P, Lorente-Galdos B, Beale H, Ramirez O, Hormozdiari F, Alkan C, Vila C, Squire K, Geffen E, Kusak J, Boyko AR, Parker HG, Lee C, Tadigotla V, Wilton A, Siepel A, Bustamante CD, Harkins TT, Nelson SF, Ostrander EA, Marques-Bonet T, Wayne RK, Novembre J. Genome sequencing highlights the dynamic early history of dogs. PLoS Genet 2014, 10: e1004016.

Freeman LM, Chandler ML, Hamper BA, Weeth LP. Current knowledge about the risks and benefits of raw meat-based diets for dogs and cats. J Am Vet Med Assoc 2013, 243: 1549–1558.

Hand M, Thatcher C, Remillard R, Roudebush P and Novotny B. Small Animal Clinical Nutrition. 5st edition. Mark Morris Institute, Topeka, Kansas. 2010.

Harper EJ, Hackett RM, Wilkinson J, Heaton PR. Age-related variations in hematologic and plasma biochemical test results in Beagles and Labrador Retrievers. J Am Vet Med Assoc 2003, 223: 1436–1442.

Harvey JW. Veterinary Hematology: A Diagnostic Guide and Color Atlas. 1st edition. W.B. Saunders, Saint Louis 2012.

Hewson-Hughes AK, Hewson-Hughes VL, Colyer A, Miller AT, McGrane SJ, Hall SR, Butterwick RF, Simpson SJ, Raubenheimer D. Geometric analysis of macronutrient selection in breeds of the domestic dog, Canis lupus familiaris. Behav Ecol 2013, 24: 293–304.

Hoffmann G, Jones PG, Biourge V, Van Den Ingh TSGAM, Mesu SJ, Bode P, Rothuizen J. Dietary Management of Hepatic Copper Accumulation in Labrador Retrievers. Journal of Veterinary Internal Medicine 2009, 23: 957–963.

Joffe DJ, Schlesinger DP. Preliminary assessment of the risk of Salmonella infection in dogs fed raw chicken diets. Can Vet J 2002, 43: 441.

Kanter MM, Kris-Etherton PM, Fernandez ML, Vickers KC, Katz DL. Exploring the factors that affect blood cholesterol and heart disease risk: is dietary cholesterol as bad for you as history leads us to believe? Adv Nutr 2012, 3: 711–717.

Kerr KR, Vester Boler BM, Morris CL, Liu KJ, Swanson KS. Apparent total tract energy and macronutrient digestibility and fecal fermentative end-product concentrations of domestic cats fed extruded, raw beef-based, and cooked beef-based diets. J Anim Sci 2012, 90: 515–522.

Khan SA, Epstein JH, Olival KJ, Hassan MM, Hossain MB, Rahman KB, Elahi MF, Mamun MA, Haider N, Yasin G, Desmond J. Hematology and serum chemistry reference values of stray dogs in Bangladesh. Open Vet J 2011, 1: 13–20.

Kovalik M, Thoday KL, Berry J, van den Broek AH, Mellanby RJ. Prednisolone therapy for atopic dermatitis is less effective in dogs with lower pretreatment serum 25-hydroxyvitamin D concentrations. Vet Dermatol 2012, 23: 125–30, e27–8.

Kronfeld DS, Hammel EP, Ramberg CF,Jr, Dunlap HL,Jr. Hematological and metabolic responses to training in racing sled dogs fed diets containing medium, low, or zero carbohydrate. Am J Clin Nutr 1977, 30: 419–430.

Lavoué R, Geffre A, Braun JP, Peeters D, Granat F, Bourges-Abella N, Trumel C. Breed-specific hematologic reference intervals in healthy adult Dogues de Bordeaux. Vet Clin Pathol 2014, 43: 352–361.

Lawrence J, Chang YM, Szladovits B, Davison LJ, Garden OA. Breed-specific hematological phenotypes in the dog: a natural resource for the genetic dissection of hematological parameters in a mammalian species. PLoS One 2013, 8: e81288.

Linder DE, Freeman LM, Holden SL, Biourge V, German AJ. Status of selected nutrients in obese dogs undergoing caloric restriction. BMC Vet Res 2013, 9: 219

Lowrie M, Garden OA, Hadjivassiliou M, Harvey RJ, Sanders DS, Powell R, Garosi L. The Clinical and Serological Effect of a Gluten-Free Diet in Border Terriers with Epileptoid Cramping Syndrome. J Vet Intern Med 2015, 29: 1564–1568.

Nielsen L, Kjelgaard-Hansen M, Jensen AL, Kristensen AT. Breed-specific variation of hematologic and biochemical analytes in healthy adult Bernese Mountain dogs. Vet Clin Pathol 2010, 39: 20–28.

Olayemi FO, Ighagbon FO. Haematology of the German Sheperd dog in a humid tropical environment. Comp Clin Pathol 2011, 20: 61–64.

Olkkola S, Kovanen S, Roine J, Hanninen ML, Hielm-Björkman A, Kivisto R. Population Genetics and Antimicrobial Susceptibility of Canine Campylobacter Isolates Collected before and after a Raw Feeding Experiment. PLoS One 2015, 10: e0132660.

Pasquini A, Luchetti E, Cardini G. Plasma lipoprotein concentrations in the dog: the effects of gender, age, breed and diet. J Anim Physiol Anim Nutr (Berl) 2008, 92: 718–722.

Peña C, Suarez L, Bautista-Castano I, Juste MC, Carreton E, Montoya-Alonso JA. Effects of low-fat high-fibre diet and mitratapide on body weight reduction, blood pressure and metabolic parameters in obese dogs. J Vet Med Sci 2014, 76: 1305–1308.

Reiter T, Jagoda E, Capellini TD. Dietary Variation and Evolution of Gene Copy Number among Dog Breeds. PLoS One 2016, 11: e0148899.

Rørtveit R, Saevik BK, Eggertsdottir AV, Skancke E, Lingaas F, Thoresen SI, Jansen JH. Age-related changes in hematologic and serum biochemical variables in dogs aged 16–60 days. Vet Clin Pathol 2015, 44: 47–57.

Schlesinger DP, Joffe DJ. Raw food diets in companion animals: a critical review. Can Vet J 2011, 52: 50.

Sharp CR, Selting KA, Ringold R. The effect of diet on serum 25-hydroxyvitamin D concentrations in dogs. BMC Res Notes 2015, 8: 442.

Skoglund P, Ersmark E, Palkopoulou E, Dalen L. Ancient wolf genome reveals an early divergence of domestic dog ancestors and admixture into high-latitude breeds. Curr Biol 2015, 25: 1515–1519.

Stiver S, Frazier KS, Mauel MJ. Septicemic salmonellosis in two cats fed a raw-meat diet. J Am Anim Hosp Assoc 2003, 39: 538–542.

Stockman J, Fascetti AJ, Kass PH, Larsen JA. Evaluation of recipes of home-prepared maintenance diets for dogs. J Am Vet Med Assoc 2013, 242: 1500–1505.

Strohmeyer RA, Morley PS, Hyatt DR, Dargatz DA, Scorza V, Lappin MR. Evaluation of bacterial and protozoal contamination of commercially available raw meat diets for dogs. J Am Vet Med Assoc 2006, 228: 537.

Swanson KS, Kuzmuk KN, Schook LB, Fahey GC,Jr. Diet affects nutrient digestibility, hematology, and serum chemistry of senior and weanling dogs. J Anim Sci 2004, 82: 1713–1724.

Torres AR. Hematologic differences between Dachshunds and mixed breed dogs. Vet Clin Pathol 2014, 43: 519.

Uhrikova I, Lacnakova A, Tandlerova K, Kucharova V, Rehakova K, Janova E, Doubek J. Haematological and biochemical variations among eight sighthound breeds. Aust Vet J 2013, 91: 452–459.

Wang GD, Zhai W, Yang HC, Fan RX, Cao X, Zhong L, Wang L, Liu F, Wu H, Cheng LG, Poyarkov AD, Poyarkov NA,Jr, Tang SS, Zhao WM, Gao Y, Lv XM, Irwin DM, Savolainen P, Wu CI, Zhang YP. The genomics of selection in dogs and the parallel evolution between dogs and humans. Nat Commun 2013, 4: 1860.

Weese JS, Rousseau J, Arroyo L. Bacteriological evaluation of commercial canine and feline raw diets. Can Vet J 2005, 46: 513.

Wennogle SA, Martin L, Oleo-Popelka FJ, Xu H, Jean-Phillipe C, Lappin MR. Randomized trial to evaluate two dry therapeutic diets for shelter dogs with acute diarrhea. Int J appl res vet med 2016, 14: 30–37.